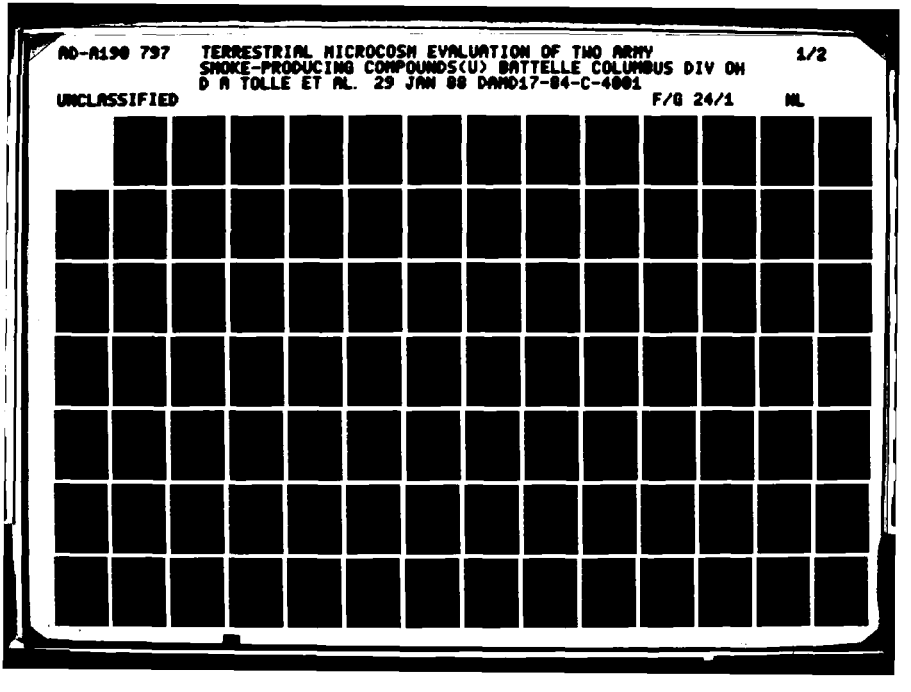
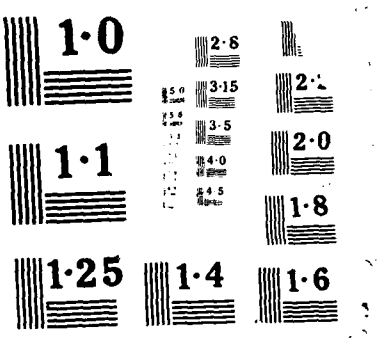
AD-A190 797 TERRESTRIAL MICROCOSM EVALUATION OF TWO ARMY SMOKE-PRODUCING COMPOUNDS(U) BATTELLE COLUMBUS DIV OH D A TOLLE ET AL. 29 JAN 88 DAMD17-84-C-4001 1/2

UNCLASSIFIED F/G 24/1 NL




1·0
1·1
1·25
2·8
3·15
3·5
4·0
4·5
2·2
2·0
1·8
1·4
1·6

(4)

AD _____

TERRESTRIAL MICROCOSM EVALUATION OF TWO ARMY SMOKE-PRODUCING COMPOUNDS

FINAL REPORT

by

D. A. Tolle, M. F. Arthur, J. Chesson, K. M. Duke,
D. R. Jackson, V. Kogan, M. R. Kuhlman, and D. P. Margeson

29 January 1988

Supported by

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5012

Contract No. DAMD17-84-C-4001

BATTELLE
Columbus Division
505 King Avenue
Columbus, Ohio 43201-2693

Project Officer: CPT (P) Gary M. Bratt, P.E., CIH
U. S. Army Medical Bioengineering Research and
Development Laboratory, Fort Detrick,
Frederick, Maryland 21701-5010

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved
OMB No 0704-0188
Exp Date Jun 30 1986

1a. REPORT SECURITY CLASSIFICATION Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION / AVAILABILITY OF REPORT Approved for public release; distribution unlimited
2b. DECLASSIFICATION / DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION Battelle Columbus Division 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code) 505 King Avenue Columbus, Ohio 43201-2693	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING / SPONSORING ORGANIZATION U.S. Army Medical Research & Development Command 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER DAMD17-84-C-4001
8c. ADDRESS (City, State, and ZIP Code) Fort Detrick, Frederick, Maryland 21701-5012	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO: 62720A; PROJECT NO: 3E162. 720A835; TASK NO: AA; WORK UNIT ACCESSION NO: 008

11. TITLE (Include Security Classification)
(U) Terrestrial Microcosm Evaluation of Two Army Smoke-Producing Compounds

12. PERSONAL AUTHOR(S)
D.A. Tolle, M.F. Arthur, J. Chesson, K.M. Duke, D.R. Jackson, V. Kogan, M.R. Kuhlman, and D.P. Margocon

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Final Report	FROM 12/15/83 TO 3/14/85	1988 January 29	148

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	Aerosol Deposition; Element Uptake; Biomass; Nutrient Loss; Ecosystem Effects; Obscurant Smokes
06	06		
07	02		

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

An intact soil-core microcosm and static exposure system were used to evaluate the potential ecological effects of two obscurant smokes--red phosphorus/butyl rubber (RP/BR) and white phosphorus/felt (WP/F), used by the U.S. Army in training exercises. Three plant species (white sweetclover, perennial rye-grass, and wheat) where chosen for preliminary and microcosm tests, because they have wide geographic distributions, can be used to reclaim disturbed ground (including Army training areas), are different physiologically and morphologically, and were shown to be capable of field representative growth in the small surface area (240.5 cm^2) of the microcosms. The preliminary stress-ethylene tests indicated that extremely high doses of either smoke would be required to elicit a response in the microcosm test. Thus, microcosms were exposed to either RP/BR or WP smoke at the following target concentrations: 0, 100, 300, 600, and 1500 mg/m^3. These doses bracketed and exceeded the typical field concentrations of 50 to 400 mg/m^3. Minor ecosystem effects were detected only at the highest smoke

20. DISTRIBUTION / AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT ☐ DTIC USERS	
22a. NAME OF RESPONSIBLE INDIVIDUAL Mrs. Judy Pawlus	22b. TELEPHONE (Include Area Code) 301-663-7325
	22c. OFFICE SYMBOL SGRD-RMI-S

DD FORM 1473, 84 MAR 83 APR edition may be used until exhausted
All other editions are obsolete

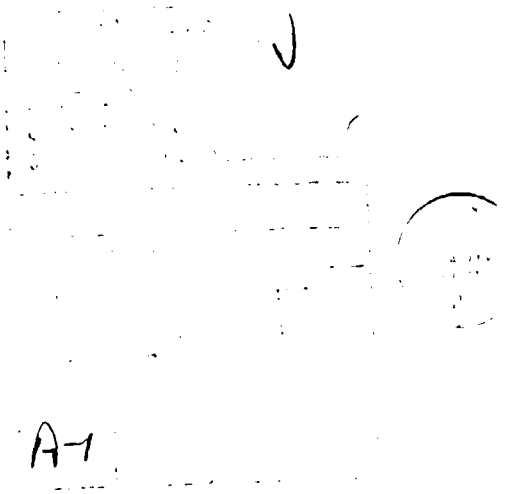
SECURITY CLASSIFICATION OF THIS PAGE

18. Subject Terms (continued)

Red Phosphorus/Butyl Rubber
Smoke Exposure Chamber
Soil Microorganism Respiration
Stress Ethylene
Terrestrial Microcosm
White Phosphorus/Felt

19. *Abstract (continued)*

concentration, including increased nutrient (Ca) loss in leachate, increased (wheat) or decreased (sweetclover) biomass yield, and increased element (Al, As, Pb, and P) uptake in plant tissue. Even the highest smoke concentrations did not affect soil microorganism respiration. No negative ecological effects of either smoke were detected at smoke concentrations equal to or below 600 mg/m^3, even for semiweekly exposures over an 8-week period. It was concluded that deployment of these two smokes at or below the above concentration and frequency was possible without significant problems to most terrestrial systems.



A-1

AD _____

TERRESTRIAL MICROCOSM EVALUATION OF TWO ARMY SMOKE-PRODUCING COMPOUNDS

FINAL REPORT

by

D. A. Tolle, M. F. Arthur, J. Chesson, K. M. Duke,
D. R. Jackson, V. Kogan, M. R. Kuhlman, and D. P. Margeson

29 January 1988

Supported by

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5012

Contract No. DAMD17-84-C-4001

BATTELLE
Columbus Division
505 King Avenue
Columbus, Ohio 43201-2693

Project Officer: CPT (P) Gary M. Bratt, P.E., CIH
U. S. Army Medical Bioengineering Research and
Development Laboratory, Fort Detrick,
Frederick, Maryland 21701-5010

EXECUTIVE SUMMARY

This report describes the evaluation of the potential ecological effects of two phosphorus obscurant smokes deployed by the U.S. Army during training activities. The technique involved use of a terrestrial microcosm and static exposure system. The objectives were two-fold: (1) to evaluate the ecosystem effects due to use of red phosphorus/butyl rubber (RP/BR) or white phosphorus/felt (WP/F) smokes, and (2) to evaluate the intact soil-core microcosm as a hazard assessment tool for Army compounds. These objectives meet two components of the broader research program designed by the U.S. Army Medical Bioengineering Research and Development Laboratory (USAMBRDL) for conducting environmental and health effects research on smokes and obscurants.

Microcosm Assessment for Phosphorus Smokes

The terrestrial microcosm system used in this study is an intact soil core, containing a natural assemblage of soil biota and an undisturbed soil profile. Thus, ecological processes occur at rates that mimic the processes and interactions of a larger ecosystem. The four ecosystem effects measured are nutrient loss in soil leachate, element uptake by plants, biomass yield, and soil microorganism respiration.

The study involved acute (a single 2-hour exposure) preliminary range-finding tests of each smoke followed by chronic (16, semiweekly, 2-hour exposures) microcosm tests of each smoke. The preliminary stress-ethylene tests determined the increase in ethylene, a sensitive measure of stress, released from potted plants due to increasing dose of RP/BR or WP smoke. This screening test has been developed by the U.S. Enviromental Protection Agency and was used to determine the appropriate dose range for the microcosm test, as well as to develop and calibrate the smoke exposure technique.

Three species of plants (white sweetclover, perennial ryegrass, and wheat) were planted in separate pots for the preliminary tests and as a mixed planting in the microcosms. These species were chosen because they have wide geographic distributions, can be used to reclaim disturbed ground (including Army training areas), are different physiologically and morphologically, and

were shown to be capable of field-representative growth in the small surface area (240.5 cm^2) of the microcosms.

Results from the preliminary stress-ethylene tests showed that very high concentrations of either smoke were required to exceed the stress-response threshold value, which is 1.5 times the mean ethylene concentration of the room-air-control plants. The threshold for the most sensitive species, sweetclover, was exceeded at the lowest smoke concentrations of roughly 18,000 mg/m^3 for RP/BR and 20,000 mg/m^3 for WP. The slopes of the dose-response curves indicated that the order of species sensitivity to either smoke from most to least sensitive is white sweetclover, perennial ryegrass, and wheat. These preliminary results, plus the knowledge that typical field concentrations are 50 to 400 mg/m^3, resulted in selection of target concentrations for the chronic microcosm test of: 0, 100, 300, 600, and 1500 mg/m^3 for each of the 16 repeat exposures. Combustion of the smoke material was performed inside the microcosm exposure chamber in order to achieve the higher target concentrations.

Results from the microcosm tests indicated that neither smoke is very toxic to terrestrial systems, except at concentrations far above typical field exposures. Minor effects were detected for nutrient loss, biomass yield, element uptake, and soil microorganism respiration at the highest target concentration (1500 mg/m^3). Regression equations for nutrient loss, biomass yield, and element uptake were based on the total phosphorus (P) deposited, because the variation between each of the 16 exposures for a given dose was lower for deposited P than for the peak aerosol mass concentration.

Nutrient Loss. Although five nutrients were monitored in microcosm leachate from the first collection, only calcium (Ca) and nitrate-nitrogen (NO_3-N) were monitored for all three collection dates. Cumulative losses were determined by summing the losses of these two nutrients from each collection date. Both regression analysis and ANOVA did not detect any significant response to either smoke for total NO_3-N. Evaluation of the quadratic term of a regression equation for cumulative Ca loss did detect a statistically significant effect ($p < 0.01$) for RP/BR smoke. The quadratic term of the regression equation was statistically significant indicating that the effect was quadratic rather than linear. There was no significant effect of WP on

cumulative Ca loss. The increased Ca loss may result from depressed Ca uptake by sweetclover, since the yield of sweetclover was also significantly decreased at the highest RP/BR treatment level. In general, legumes have considerably more Ca, as percent of dry matter, than grasses.

Biomass Yield. Biomass yield (above-ground, oven-dry weight) from each of the two harvests was analyzed separately and as a combined total for both harvests. In the second harvest, biomass for each species was analyzed separately and as a combined total for all species. Analysis of the linear and quadratic terms of regression equations, as well as ANOVA, did not detect any significant effect due to exposure to either smoke for total biomass from the first harvest, total biomass from both harvests combined, or ryegrass biomass from the second harvest. Significant effects were only found for sweetclover biomass (second harvest) due to exposure to both smokes and wheat biomass (second harvest) due to exposure to WP smoke.

Bonferroni's test on sweetclover biomass data from the second harvest indicated that sweetclover biomass at the 1500 mg/m^3 dose was significantly ($p < 0.05$) lower than controls for both smokes, while the regression trend for wheat biomass exposed to WP smoke suggested an increase in biomass at the 1500 mg/m^3 dose relative to controls. The opposite effect of the high smoke dose on these two species may be a result of their differing sensitivities to arsenic (As), which is a contaminant in both smokes. Legumes, including sweetclover, are very sensitive to As, while wheat has been stimulated by low application rates of calcium arsenate.

Element Uptake. Data on plant uptake of 24 elements from the first harvest were analyzed statistically, in order to make a decision on which six elements would be chemically analyzed in the second harvest. Based on these analyses, plus data in the literature on the relative toxicity of these elements, the following six elements were analyzed in plant tissue from the second harvest (Al, As, P, Pb, Cr, and Mo).

Statistical analysis of plant concentration data from the second harvest showed significant effects of one or both smoke on As, P, Al, and Pb uptake by plants. Plant uptake of Al and Pb was significantly affected ($p < 0.05$) by WP smoke, based on the linear term of the regression equation.

Significant effects of both smokes on As and P uptake by plants was detected by both the linear term of a regression equation ($p < 0.001$) and ANOVA ($p < 0.05$). Neither the equations from regression analysis nor ANOVA detected any significant ($p > 0.05$) effects of exposure to either smoke on Cr or Mo uptake or of exposure to RP/BR smoke on Al or Pb uptake. Uptake of Al, As, and Pb by plants exposed to the phosphorus smokes may be the result of their presence as impurities in the unburned phosphorus material used in the smokes. Levels of these elements in unburned WP/BR reported in another study were: Al-20 ppm, As-84 ppm, and Pb-1.27 ppm. The levels of these elements in the unburned WP and RP/BR used in this study were not determined.

Of the four elements (As, Al, Pb, and P) which showed significant uptake in plant tissue, only As had the potential to affect biomass yield. Arsenic may have depressed sweetclover biomass at the high treatment level of both smokes and increased wheat biomass at the high treatment level of WP smoke. None of the element uptake data indicated any potential impact on grazing animals due to toxic element concentrations in plant tissue. The addition of As to the soil may be responsible for the significant increase in Ca loss in leachate from RP/BR-smoke-treated microcosms, since toxic element application to terrestrial microcosms in other studies has resulted in increased nutrient loss.

Soil Respiration. Exposure of the soil-core microcosms to RP/BR or WP smoke had almost no measurable impact on the ability of microorganisms to mineralize added organic substrate with resultant evolution of CO_2-C. The rate of CO_2-C evolution from microcosm soil was analyzed by a one-way ANOVA and quadratic or linear regression on target dose for both smokes. The ANOVA detected no statistically significant effect ($p > 0.05$) of either smoke on the rate of CO_2-C decline. The quadratic term from regression analysis did detect a statistically significant effect ($p < 0.05$) of RP/BR smoke, suggesting a slightly increased microbial activity at the target dose of 600 mg/m^3. The same analysis detected no significant effect of WP smoke.

Microcosm Efficacy

The terrestrial microcosm and static exposure chamber system produced positive dose-response curves for ecological effects with two, low-toxicity aerosols (RP/BR and WP smoke). Thus, the system appears to be appropriate for evaluating the static, long-term particle-deposition effects of other aerosols. The negative ecosystem effects of these two smokes, however, were elicited only at smoke exposure levels far above those expected during field training exercises. Also, it is not known whether plant effects would have been elicited at even lower exposure levels, if a dynamic exposure system was used that involved wind-driven impacts, including smoke particle impact on the down side of leaves.

The test design used in this study adequately produces input for hazard assessment at a considerable savings in cost and time compared to the more traditional range-finding plus definitive test strategy using microcosms for both tests. In this study, the plant stress-ethylene test provided information on the appropriate dose range for microcosm testing and the microcosm test combined aspects of both the traditional range-finding and definitive tests using microcosms. This combination of range-finding and definitive into one test is the approach adopted by the U.S. EPA for their current Level 1 environmental assessment biological tests.

Both RP/BR and WP/F appear to cause no significant negative ecological effects on terrestrial ecosystems at concentrations equal to or less than 600 mg/m^3, even for semiweekly exposures over an 8-week period. Thus, deployment of these two smokes at or below the above concentration and frequency is not expected to result in significant problems to most terrestrial systems. Soils considered potentially sensitive to acid precipitation (i.e., circumneutral, noncalcareous soils with low cation exchange capacity) may be the exception. These soils have been mapped by acid rain research.

FOREWORD

The U.S. Army Medical Research and Development Command (USAMRDC) is conducting environmental and health effects research to develop the biomedical data base required to establish criteria/standards for exposure of troops, industrial workers, and the environment to chemicals associated with smoke/obscurants production, use, and disposal. The research reported here fulfills one component of USAMRDC's environmental research on two phosphorus obscurant smokes, red phosphorus/butyl rubber (RP/BR) and white phosphorus felt (WP/F). The environmental effects of these two smokes were evaluated using a terrestrial microcosm and static exposure system.

Citation of trade names in this report does not constitute a Department of the Army endorsement or approval of the use of such items.

The close cooperation of Mr. Jesse J. Barkely, Jr. and CPT (P) Gary Bratt of U.S. Army Medical Bioengineering Research and Development Laboratory (USAMBRDL) is gratefully acknowledged. The authors would like to acknowledge all of the personnel who worked on this project that are listed at the end of the report under Publications and Personnel. Special thanks are due to technicians Thomas C. Zwick and G. Kelly O'Brien, who assumed the lion's share of plant maintenance and microcosm exposure activities.

TABLE OF CONTENTS

TABLE OF CONTENTS
(Continued)

TABLE OF CONTENTS
(Continued)

LIST OF TABLES

LIST OF TABLES
(Continued)

LIST OF TABLES
(Continued)

LIST OF FIGURES

LIST OF FIGURES
(Continued)

FINAL REPORT

TERRESTRIAL MICROCOSM EVALUATION OF TWO ARMY SMOKE-PRODUCING COMPOUNDS

by

D. A. Tolle, M. F. Arthur, J. Chesson, K. M. Duke,
D. R. Jackson, V. Kogan, M. R. Kuhlman, and D. P. Margeson

31 October 1984

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5012

Contract No. DAMD17-84-C-4001

Battelle's Columbus Laboratories

1.0 INTRODUCTION

1.1 Statement of the Problem

Many U.S. Army training and testing activities release smoke and obscurant chemicals to the environment and, thus, have the potential for adverse environmental effects. The Army is committed to the reduction, mitigation, or control of environmental effects from Army training activities. As a result, the U.S. Army Medical Research and Development Command (USAMRDC) is conducting environmental effects research to develop the data base required to establish criteria/standards for exposure of the environment to chemicals associated with smoke/obscurants production, use, and disposal.

One component of USAMRDC's environmental research on smokes is the evaluation of ecosystem effects due to the use of red phosphorus/butyl rubber (RP/BR) or white phosphorus/felt (WP/F) smokes during training activities. Research completed to date has dealt primarily with the chemistry and/or toxicology of these smokes (e.g., references 1, 2, 3, 4). The microcosm technique used in this study is designed to evaluate the ecosystem-level effects on terrestrial systems due to deployment of RP/BR or WP/F, and thus fill a major gap in the environmental evaluation of these smokes. At the same time, refinement of the microcosm technique to include testing of aerosols will permit more efficient and cost-effective evaluation of other Army smokes.

The two military obscuration smokes (RP/BR and WP/F) evaluated in this study are used as hasty projected smokes designed to provide a protective screen while troop and/or equipment movement takes place. Both of these projected screening smokes produce a dense white smoke containing a series of polyphosphoric acids[2] when ignited (RP/BR)[1] or exposed to air (WP/F)[3]. The smokes are used in Army training activities resulting in significant environmental exposure and the potential for adverse effects.

Both the WP/F and RP/BR smokes consist primarily of a series of polyphosphoric acids, especially orthophosphoric acid (H_3PO_4) and pyrophosphoric acid ($H_4P_2O_4$), but also higher members of the series up to polymer number 8[1,3]. Trace impurities in the unburned WP include numerous elements, particularly boron, silicon, iron, and arsenic, as well as smaller concentrations of aluminum and lead[3] Organic constituents associated with the combustion of the WP/F smoke munition included part per billion levels of methane, ethylene, carbonyl sulfide, acetylene, 1,4 dicyanobenzene, 1,3-dicyanobenzene, 1,2-dicyanobenzene, acetonitrile, and acrylonitrile. Commercial RP contains trace impurities, such as iron, copper, silver, bismuth, and nickel[1,2]. Thus, chemical characteristics of the two phosphorus smokes are similar.

1.2 Background on Microcosm Technique

Environmental legislation over the past two decades has stimulated the development and refinement of biological testing procedures. These laws require the assessment of biological hazards associated with chemicals and wastes released to the environment. The protocols developed for these hazard assessments usually incorporate widely accepted, single species bioassays, which focus on the organism and population components of ecological systems. In the past, multi-species tests designed to evaluate community- and ecosystem-level effects were omitted. This absence has been recognized and considerable research effort has been directed toward the development of procedures for assessing both community- and ecosystem-level effects of chemicals and wastes in the environment. The microcosm approach has proven to be one of the most promising tools resulting from this research[5,6,7].

Microcosms are confined portions of an ecosystem (aquatic or terrestrial) under laboratory control that attempt to mimic the processes and interactions of a larger ecosystem[6]. The investigator has control over environmental variables, such as temperature, humidity, light, and water. Microcosms may be either artificially fabricated (i.e., carefully assembled in the laboratory from either natural or man-made components), or intact pieces of natural ecosystems brought into the laboratory. Terrestrial microcosms with intact soil cores, such as the type used in this study, are thought to be an improvement over artificially constructed terrestrial microcosms, because they contain a natural assemblage of soil biota and an undisturbed soil profile[7,8]. Thus, ecosystem-level processes, such as cycling of plant nutrients, element transport and fate, plant-microbial interactions, and soil microsite chemistry occur at natural rates.

Considerable developmental work has been done with terrestrial microcosms since 1979[9,10,11,12]. The microcosm resulting from this developmental work is a medium-sized, intact soil core containing producer, decomposer, mycorrhizal, and root components in the soil matrix[13]. These cores are maintained in a greenhouse under natural or controlled photoperiods (depending on the time of year) with rainfall input simulated. Parameters monitored

include nutrient loss in leachate from the core, biomass yield, element uptake in plants, and soil microorganism respiration. The rate of loss of nutrients such as nitrate-nitrogen from the soil core is an effective indicator of the overall response of the system[14,15]. If these complex interactions of biotic and abiotic components are disrupted by addition of toxicants, increased loss of nutrient loss in soil leachate will eventually lead to a decrease in plant growth. Thus, nutrient loss is an integrating measure influenced by both physicochemical and biological processes. Biomass yield, the second parameter monitored, is also used as a measure of ecosystem response. The above-ground portion of vegetation is exposed to the gaseous/particulate phase of a chemical or waste while the root system is affected directly by the solids deposited on the surface of the microcosm soil or by amendment-induced changes in soil chemistry.

The third parameter monitored in microcosms is element uptake by plants. Here, the fate of potentially toxic elements, especially metals, present in the waste are monitored. Microcosms have been used to investigate the fate of potentially toxic elements in addition to assessing element effects on ecosystem processes[8,16,17]. These studies showed that many toxic trace elements are distributed in microcosms in a manner very similar to the field situation.

A fourth ecosystem parameter monitored in connection with microcosm testing is a soil respiration test using waste-contaminated soil from the microcosms at the completion of other tests[18]. This test involves the measurement of carbon dioxide-carbon (CO_2-C) evolved from waste-treated versus control soils. The results from this test can be used to verify the phytotoxic effects of a waste material as determined by mirocosm-grown plants.

1.3 Objectives

The objectives of this research are two-fold: (1) to evaluate the applicability of the intact soil-core microcosm as a hazard assessment tool for evaluating ecosystem-level effects of chemicals (including aerosols) used by the Army, and (2) to evaluate the ecological effects (i.e., nutrient loss, element uptake, biomass yield, and soil microorganism respiration) of RP/BR and WP/F smoke using the terrestrial microcosm. Both of these objectives

require development of the aerosol generation and exposure systems for microcosm dosing, which will facilitate future microcosm testing of Army smokes/obscurants.

2.0 MATERIALS AND METHODS

2.1 Overall Study Design

This project was organized according to four tasks: Task 1, Exposure; Task 2, Ecology; Task 3, Analytical Chemistry; and Task 4, Biostatistics. Task 1 involved the development of smoke generation systems and exposure of soil-core microcosms to smokes at predetermined concentrations. Task 2 included the construction, maintenance, and monitoring of terrestrial microcosms dosed with the various concentrations of smoke. This task included an initial screening test for effects on plants due to the obscurant smokes by employing a stress ethylene test. Exposure of soil-core microcosms to the smokes subsequently was based on preliminary stress ethylene results. Task 3 involved the physicochemical characterization of combusted smokes, as well as characterization of the soil leachate changes from application of deposited smoke aerosols on soil. This task also included analyses of plant tissue for potential changes in element uptake. Finally, Task 4 involved both the statistical design of the study and statistical analyses of the resultant data. To more clearly describe the overall study design, the following two sections discuss the biological and statistical approaches, respectively, used to accomplish the project objectives.

2.1.1 Biological Design

The basic objectives of this program were to expose plants grown in soil-core microcosms to various concentrations of RP/BR and WP smokes, and then to determine what effects the smokes had on the soil-plant system. Initially it was decided to aim for smoke concentrations that were environmentally relevant, as well as higher and lower concentrations. Also, the use of plant species with relatively widespread habitats was considered important because of the potential for Army training exercises to occur at a number of geographical locations. Selection of the parameters for determining the environmental effects of the obscurant smokes was based on the need to monitor the soil-plant system in both a complete and efficient manner.

Three species of plants were chosen for the microcosm exposures based on germination trials of six species (see Section 2.2.2). A stress ethylene test was used as a preliminary screening test (see Section 2.2.4) for smoke exposure. The stress ethylene test was chosen based on earlier observations that the plant hormone, ethylene, which is produced normally at very low levels by plants, is produced in excess when a plant is chemically or physically stressed[19,20]. The amount of ethylene released is proportional to the degree of stress over a fairly wide range of doses. Very toxic conditions, however, can cause tissue death and thus lower the levels of ethylene released compared to controls.

Once a series of stress ethylene tests was completed using the three plant species selected earlier, the results were used to select five target doses of smokes for exposure to the soil-core microcosms in the microcosm tests (see Section 2.4.1). Concomitantly, five special exposure chambers were constructed so that each one fit over a microcosm cart containing three replicate microcosms (see Section 2.4.2). Sixty intact soil-core microcosms that had been extracted from an undisturbed (for many years) field site were set up in a greenhouse under strict temperature, humidity, and lighting control (see Section 2.4.7). The cores were planted with the same three species used in the stress ethylene tests. The 60 cores were divided equally between two greenhouse bays, 30 cores for exposure to RP/BR and 30 cores for exposure to WP. Within each group of 30 soil-core microcosms six replicate cores were designated for each target dose. Because each microcosm cart was constructed to hold three soil-cores, this gave two carts per target dose. The rationale for the statistical design is explained in detail in Section 2.1.2.

Each group of three microcosms was exposed on 2 days of a week for 8 weeks. On Monday and Wednesday mornings, one group (A) of cores were exposed to WP. On Tuesday and Thursday mornings, a second group (B) of cores were exposed to WP. A similar weekly exposure pattern occurred for RP/BR, but these exposures were all done in the afternoon (see Section 2.4.4). On Fridays following exposures, leaf surfaces were sprinkled with water to simulate rainfall washing of leaves (see Section 2.4.7). Monitoring of environmental parameters continued for 1 weeks post-exposure. For monitoring purposes, microcosms were harvested two different times through the growing period and the dry biomass yields were determined (see Section 2.4.8.2).

Uptake of 24 elements in the biomass from the first harvest and six elements in the biomass from the second harvest was determined. Soil-core leachates were collected three times (see Section 2.4.8.1). The first leachate was analyzed for five nutrients, while the second and third leachates were each analyzed for two nutrients.

At the completion of the smoke exposures and subsequent monitoring, soil from the microcosms was monitored for CO_2 evolution in a soil respiration test, as a test for effects on soil microbial communities (see Section 2.5). These and other data were handled statistically in order to identify significant effects. The overall statistical design is outlined in the following section.

2.1.2 Statistical Design

The design of the microcosm test was based on the results of the preliminary screening bioassays which indicated that RP/BR and WP/F smokes are not likely to cause large responses even at relatively high exposure levels. The microcosm tests were designed to provide reliable confirmation of this observation. For each smoke there were five target exposure levels. Microcosms were exposed in groups of three because it is impractical to have a separate exposure hood for each microcosm. This imposes a dependence between the three microcosms exposed together in the same chamber which is not present between microcosms exposed in different chambers. The problem of pseudoreplication has been discussed in detail by Hurlbert[21]. Microcosms from a single exposure chamber are likely to be more similar than those from different chambers and this must be taken into account in the statistical analysis. This potential problem was alleviated by having two groups of three microcosms for each exposure level, thus introducing between, as well as within, chamber variation. Information from each group of three was pooled, either as part of the experimental procedure (e.g., pooling leachates), or by averaging the three values (e.g., biomass), to give two independent replicate values per exposure level. The pooling reduces the overall error variance by averaging over individual microcosms and avoids the problem of incorrectly using individual microcosms as independent observations.

2.2 Preliminary Tests

As indicated in Section 2.1, a number of preliminary tasks were conducted prior to the major task of exposing soil-core microcosms to obscurant smokes. These preliminary tasks comprised two important areas: development of exposure methods, and stress ethylene testing. They served to identify reasonable exposure doses, methods, and frequency. Thus, subsequent microcosm exposures were conducted with a minimum of complications, problems, and costs. Preliminary procedures included exposure techniques for both smokes, plant selection for species and age, plant seeding and maintenance, stress ethylene collection and analysis, and observations of plants for signs of smoke-induced stress. These areas are described in detail in the following sections.

2.2.1 Exposure Techniques for Both Smokes

The exposure techniques used for the preliminary tests were the same for both RP/BR and WP/F, but did vary depending upon the desired dosage for the plants. For all exposures, a two-hour exposure duration in the mixed chambers was used--this being the elapsed time from the start of aerosol generation to the start of chamber exhausting. The aerosol generation period typically lasted up to 2 minutes for the RP/BR, and up to about 30 seconds for the WP/F. For all exposures the plants were inserted into the small chambers (see Section 2.2.4), which were then closed prior to aerosol generation. The plants were carefully removed from the chambers after the smoke was exhausted from the chamber.

To achieve the lowest plant dosage in these tests, a definite amount of smoke-producing material was ignited in a combustion flask attached to the intake line of a chamber, while the exhaust line was connected to the suction outlet of a vacuum pump. Thorough mixing of the chamber atmosphere was provided by a built-in recirculation fan. Both inlet and outlet valves were shut off at the end of the combustion period and the exposure period was initiated at this point. An air sample taken with a 20-ml syringe at the beginning of exposure was injected, following repeated purging of the syringe with chamber aerosol, into a 3.72-ℓ dilution box connected to the Respirable Aerosol Mass

Monitor (Model TSI 3500-PIEZOBALANCE) for measuring aerosol mass concentration. The magnitude of the smoke concentration measured at the beginning of the exposure was used to characterize the exposure level.

To achieve higher dosages of both smokes, a different aerosol generation technique was employed. In this method, a measured mass of smoke-producing material was ignited in a combustion dish which was quickly placed on a supporting plate in the upper portion of an exposure chamber. Exposure time was started at this point, and the level of exposure was taken to be the maximum aerosol concentration detected during the combustion period.

For the highest dosages, multiple (up to four) aerosol injections into the static chambers were made in order to overcome the fairly rapid decay of the airborne aerosol concentration. Since the applied dosage is the amount of aerosol phosphorus mass deposited on the plants, these multiple injections afforded a means of multiplying the dosages with relatively good control. Each aerosol injection was equivalent to a smoke concentration of roughly 20,000 mg/m^3 for WP/F and 18,000 mg/m^3 for RP/BR (see Appendix A, Figures A-1 and A-2).

2.2.2 Selection of Plant Species and Test Age

Army training maneuvers with smokes may be carried out in a large variety of habitat types throughout the United States. Therefore, plant species used in ecological effects testing of obscurant smokes should be fairly common to large parts of the country. At the same time, the plants should be capable of field-representative growth within the confines of the small surface area (240.5 cm^2) of the soil-core microcosm. Grasses ultimately chosen for testing in the stress ethylene and the microcosm tests included perennial ryegrass (Lolium perenne) and wheat (Triticum laestivum); a legume, white sweetclover (Melilotus alba) was also chosen. These species were selected after conducting comparative seed germination tests with tall fescue (Festuca arundinacea), oats (Avena sativa), and birdsfoot trefoil (Lotus corniculatus). The final selection of plants included a grass important in land reclamation (perennial ryegrass), a legume (nitrogen fixer) also used in reclaiming disturbed land (white sweetclover), and a food crop (wheat) for evaluating element uptake in a species used for human consumption.

To conduct the seed germination tests among the six species each species was planted in flower pots of potting medium maintained in a growth chamber at 25°C with a 16-hour/8-hour light/dark regime. Pots were watered daily with reverse-osmosis-(RO) treated water and monitored for percent germination, plant vigor, and plant height. Following the selection of the three test species (as described above) from the germination trials, 40 pots of each species were planted weekly and maintained in the greenhouse. After germination, plants were thinned to 20 plants per pot, watered daily, and monitored for growth. The weekly planting and monitoring allowed for the selection of plants of relatively uniform development for subsequent stress ethylene testing. Twenty-seven-day-old sweetclover and ryegrass, and 13-day-old wheat provided uniformly developed plants.

2.2.3 Plant Seeding and Maintenance

Following the determination of the appropriate plant age, 40 pots of each species were planted weekly as described above for use in the stress ethylene test and were watered daily with half-strength Hoagland's nutrient solution.

Content of Hoagland's Nutrient Solution

Chemical	g/l
KH_2PO_4	0.036
KNO	0.505
$Ca(NO_3)_2$	0.820
$MgSO_4$	0.241
	mg/l
H_3BO_3	2.86
$MnCl_2 \cdot 4H_2O$	1.81
$ZnSO_4 \cdot 7H_2O$	0.22
$CuSO_4 \cdot 5H_2O$	0.08
$H_2MoO_4 \cdot H_2O$	0.02
Iron tartrate	5.0

This provided a constant stock of plants of the appropriate age for several trials of the stress ethylene test. During this period, greenhouse bays were monitored constantly for humidity and temperature using calibrated chart recorders and visual observation of monitoring equipment. Relative humidity

was maintained generally between 65-85 percent by the use of supplemental high-output humidifiers. Temperature regulation to maintain close to the optimum of 25°C required daily attention to switch from steam heat to the use of swamp coolers on sunny days.

2.2.4 Ethylene Collection and Analysis

The stress ethylene test described by Brusick and Young[22] and Thompson et al.[23] was used to determine the lowest concentration of phosphorus smokes required to elicit plant stress. This screening test is based on the increase in ethylene production (a normal plant hormone) by plant tissue as a function of increasing physical or chemical stress.

Stress ethylene exposure chambers were 61 cm wide by 56 cm deep and 76 cm high, with a 19-mm plywood frame and 1-mil Mylar film taped to the frame with duct tape for sides and top. To provide internal air circulation in the chambers during exposure, thus preventing concentration gradients from developing in the chamber, an internal air circulation system was added to each chamber. A small blower was mounted below the chamber's peg-board false bottom and was connected to a vertically mounted 31.8-mm-diameter polypropylene pipe above the false bottom. The blower draws in air from the area beneath the false bottom and exhausts it through the pipe into the area above the false bottom. The motor driving the blower was mounted outside the chamber to prevent ozone and hydrocarbons (volatilized lubricants) from entering the chamber and influencing the test results.

Phosphorus test materials (RP/BP and WP/P) were combusted directly in the chambers (see Section 2.2.1) so no special intake manifolds were required for the stress ethylene chambers. Chlorine gas was used as a positive control and was injected by syringe into a positive control stress ethylene chamber. The chamber exhaust system consisted of a 19-mm-diameter polypropylene pipe, an on-off ball valve, an orifice type calibrated flow meter, and a regulating valve, all connected to the exhaust manifold. A high pressure Cadillac 20,000-rpm blower was used in the exhaust system to draw the samples being tested from the chamber and through an activated charcoal filter.

In preparation for the stress ethylene test with RP/BR and WP/F, a rough approximation of ethylene response by the three plant species were evaluated by a phosphoric acid spraying test. Five concentrations (0.0, 0.5, 1.0, 5.0, and 10.0 percent) of H_3PO_4 were applied to plants using an atomizer. Of the 5 mL sprayed on each flower pot under a hood, about 2-3 mL stayed on the plants. One flower pot of each of the three plant species was tested for each concentration. These pots were sealed individually into polyethylene bags with wire supports to keep the leaves from touching the bag. Any visually obvious effects (lesions, discoloration, wilting) were noted at this time and the enclosed plants were placed in darkness for 5°C for a 24-hour incubation during which released ethylene accumulated inside the bags. Dark incubation was to stop the photosynthesis process and prevent O_2 depletion during the 24-hour period.

To determine the quantity of ethylene produced, a gas-tight syringe was used to puncture the polyethylene bag and withdraw approximately 500 mL of gas after the incubation period. Ethylene was determined by co-chromatography with standard ethylene using a Varian 3700 gas chromatograph. Only the ryegrass showed a clear ethylene response. The peak ethylene response for ryegrass was at the 0.5 percent H_3PO_4 treatment level. The leaves on all three plant species were severely damaged or killed at the two highest H_3PO_4 treatment levels. The high concentration treatments very likely damaged the ethylene production mechanism and prevented an ethylene response. Although this preliminary test was not meant to be conclusive, it did indicate that the stress ethylene test using smokes should be designed to evaluate concentrations roughly equivalent to 0.5 percent phosphoric acid.

The stress ethylene test using RP/BR and WP/F smokes was conducted in a similar fashion. Three pots of each plant species were placed in a test chamber and exposed to smoke as described in Section 2.2.1. Each treatment level consisted of one to four repeat combustions of aerosol, equivalent to roughly 20,000 mg/m^3 for WP/F and 18,000 mg/m^3 for RP/BR at each injection. External supplemental lighting was provided using sodium vapor lamps in the greenhouse. After two hours of exposure, the chambers were exhausted, the plants were removed and individually bagged and were incubated at 25°C for 24 hours in the dark. Ethylene was sampled and analyzed as described above for the phosphoric acid test.

2.2.5 Plant Necrosis Observations

All plants were monitored visually for lesions, discoloration, and wilting during the exposure period and immediately after the subsequent incubation for ethylene collection. In addition, all plants were monitored visually for several days after the ethylene collection to note any lethal effects due to smoke exposure.

2.3 Soil Leachate Characterization

Soil of the type used in the microcosm study was leached with aqueous extracts of smoke aerosols in order to discern possible physicochemical changes in leachate composition. This procedure was desired because of the possibility of improperly attributing potential changes in leachate composition from microcosms to biological effects when in fact the observed change might be caused by physicochemical processes, such as ion exchange with soil.

Soil leachate was obtained by using a column leaching procedure recently developed by Jackson et. al.[24]. This procedure consists of mixing 100-g samples of air-dry, sieved soil with an equal mass of Ottowa sand. The sand-soil mixture is placed into glass cylinders having adjustable column inserts. An additional 15-cm layer of sand is added to prefilter the soil leachate. Loaded soil columns are leached with a total of 1 liter of distilled water over an 18-hour time period.

Time-regulated rotary valves apportion the leachate into three equal subfractions of approximately 333 ml each. Each soil leachate fraction was filtered through 0.45 μm membranes. Aliquots of leachate were then submitted for chemical analysis. Chemical analyses included K, Ca, NH_4-N, NO_3-N, and on selected samples, total organic carbon (TOC) (see Section 2.4.8.1 for nutrient analysis methods).

Samples of smoke aerosols deposited on petri dishes were dissolved in 50 mL of distilled water. A volume of 6.13 mL of this extract was applied to each treated soil column. This volume represented an equivalent dose rate on the soil columns as that received on the deposition coupons.

The experimental design consisted of leaching WP and RP/BP from deposition coupons exposed to smoke aerosols at target concentration rates of

0, 100, 1500, and 20,000 mg/m^3. These doses were chosen to represent the low (100 mg/m^3) and high (1500 mg/m^3) target doses planned for the range-finding test, and the minimum dose (20,000 mg/m^3) required to produce a response in the stress ethylene screening test. The experimental design was replicated in duplicate. The dosage aliquots were applied to the soil column by immersing the pump inlet into a vial containing the dissolved aerosol sample. This procedure was immediately followed by immersing the pump inlet into a distilled water reservoir for continuing the leaching procedure.

2.4 Microcosm Tests

Based on the relatively low toxicity of the phosphorus smokes as determined by the preliminary tests, it was decided to conduct an expanded range-finding test that contains some elements of both the traditional short-term range-finding test and the very long-term definitive test. The resulting test design involved 2-hour-long, semiweekly exposures of microcosms to RP/BR or WP smoke over an 8-week period. Monitoring of microcosm parameters was conducted during and after the exposure period, for a total of 12 weeks. The exposure methods and microcosm maintenance and monitoring techniques for the microcosm test are described in the following paragraphs.

2.4.1 Selection of Smoke Treatment Concentrations

Target smoke concentrations for exposure of microcosms in the microcosm test were based on results of the plant stress-ethylene test and realistic field exposure levels. The minimum concentration of either smoke required to elicit a response in the plant stress-ethylene tests was approximately 18,000 to 20,000 mg/m^3. On the other hand concentrations of WP smoke measured during a research project in the field reached only 7 mg/m^3[25]. Typical concentrations of both phosphorus smokes during field training exercises are estimated by USAMBRDL staff to be in the neighborhood of 50 to 400 mg/m^3. Therefore, the smoke concentrations selected for the range-finding tests were between the minimal level (7 mg/m^3) determined in field research and the extremely high levels (18,000 to 20,000 mg/m^3) required to obtain a plant stress-ethylene response. The target dose levels chosen for both smokes were 0, 100, 300, 600, and 1500 mg/m^3, which bracketed typical field concentrations, yet were spread far enough apart that deviations of actual exposures

from two adjacent target doses were not likely to overlap. It was assumed that biweekly doses of 1500 mg/m^3 over 8 weeks would be far above the continuous field exposure levels at any Army installation.

2.4.2 Exposure Chamber System

The exposure chamber system used in the microcosm tests is depicted in Figure 1. The chamber itself is a bottomless Plexiglas® cube, 120 cm on a side. The bottom of the cube is supplied by the microcosm cart surface onto which the chamber is lowered for exposure performance. Four chambers were constructed and used for actual smoke exposures, and a fifth one was used for the control microcosms. Each exposure chamber was equipped with a low rpm mixing fan to insure a homogeneous atmosphere, a thermocouple for gas temperature measurement, a hygrometer to provide continuous indication of the chamber relative humidity, an exhaust port which led to an outside stack, an injection/entry port, and an aerosol sampling port. Each exposure chamber was also provided with external cooling by ventilation fans to prevent heat-up of the chamber by the plant growth lamps suspended above them.

2.4.3 Removal of WP from Felt Matrix

Preliminary testing of the WP/F munition indicated that reproducibility of smoke production with this material would be quite difficult. Sections of the WP/F wedges were used in these initial tests, and gave highly variable results. Visual inspection of the sections prior to use showed that they appeared to contain differing amounts of WP. To achieve consistency in the amount of WP burned for each test, the WP was extracted from the felt matrix of the munition. This was accomplished by immersing the wedges in hot (60 C) water to melt the WP and squeezing this liquid out of the felt. Measured volumes (0.1 mL) of the liquid WP were then pipetted into a mold which was placed in cold water for solidification of the pellets. After refreezing of the WP, the pellets were easily removed from the mold (underwater) and placed for storage in small jars of water. This process yielded reproducible pellets of WP which were easily ignited for the aerosol generation.

reproducible pellets of WP which were easily ignited for the aerosol generation.

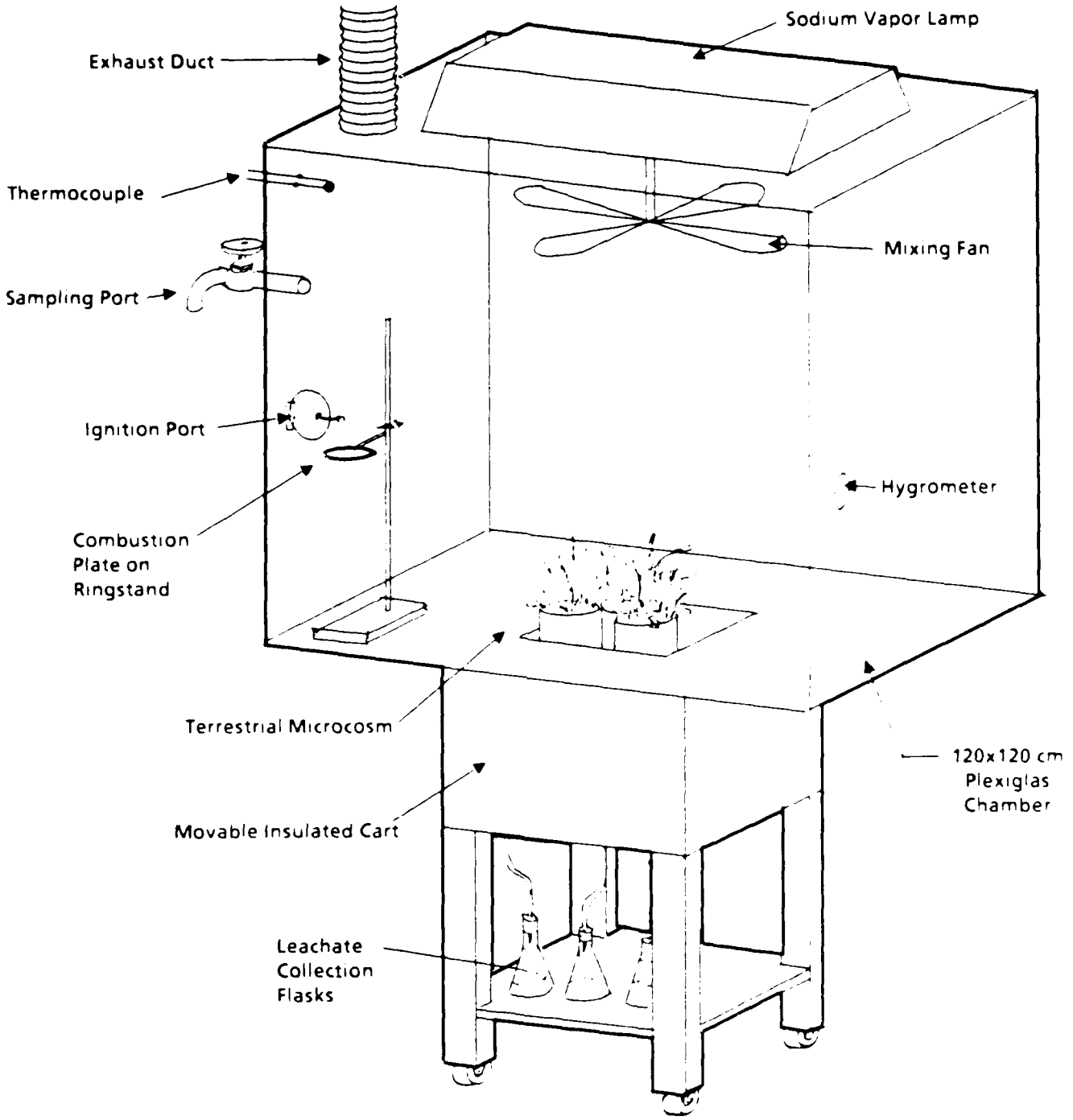


FIGURE 1. EXPOSURE CHAMBER SYSTEM FOR DOSING MICROCOSMS IN MOVABLE CARTS

2.4.4 Exposure Protocol for Both Smokes

Based upon the results of the stress-ethylene tests in the preliminary experiments performed and typical field concentrations (see Section 2.4.1), the following initial target concentrations of smoke aerosol were selected for the exposure levels in the microcosm testing portion of the study: 0, 100, 300, 600, and 1500 mg/m^3. To achieve these initial concentrations, a single aerosol injection was performed by igniting a measured mass of RP/BR or WP on a platform in the exposure chamber. Care was taken to avoid any possibility of direct contact of the burning material with the plant surfaces. Temperatures within the chambers were also monitored to ensure the absence of excessively hot conditions for the exposures. The ignition typically required seconds, and combustion from 30 seconds to as much as 10 minutes, depending upon the material and the amount of mass burned.

Following the combustion period, the initial aerosol mass concentration was measured by withdrawing a sample of the chamber atmosphere, diluting it, and measuring the concentration as described below. The temperature and relative humidity in each chamber was recorded intermittently during the exposure. At an elapsed time of two hours from the start of the combustion of RP/BR or WP, the chamber exhaust port and intake were opened and the chamber atmosphere was rapidly replaced with room air. A more detailed description of the run protocol is found in Appendix A.

2.4.5 Exposure Measurements

The exposure characterization consisted of measurement of the environmental variables, the initial aerosol mass concentrations, and the deposited phosphorus mass. This latter parameter was indicative of the actual dosage applied to the plants. The chamber temperature and relative humidity were monitored by a chromel-alumel thermocouple and a hygrometer, respectively. The initial mass of material to be burned for aerosol generation was weighed on a balance, for the RP/BR, or was determined by using a specified number of identical WP pellets (0.1 mL/pellet; see Section 2.4.3).

The initial aerosol concentration was measured to provide an indication of the potential dose received by the microcosms, and to provide a measurement which could be used to relate the experimental exposure levels to

those measured under field conditions. From this data, the exposure chamber concentration was calculated. These measurements were performed by withdrawing a small aliquot of the chamber atmosphere using a gas-tight volumetric syringe, and injecting it into a mixed container of known volume to dilute the sample. The TSI Model 3500 Respirable Mass Monitor then sampled from this dilution box for a measured time interval to determine the mass concentration in the box. This instrument measures aerosol mass concentration by pulling a known volume of air thorugh a chamber in which the aerosol particles are deposited electrostatically upon a piezoelectric crystal whose frequency of vibration is monitored electronically. This frequency shift is converted into a mass concentration. The principle of operation of this instrument has been described in more detail by Sem and Tsurubayashi[26]. Factory calibration of this device involves comparison of the frequency shift of the instrument's crystal against mass concentrations determined using filter samples and gravimetric analysis. This calibration is valid provided the instrument is operated within the range of conditions identified by the manufacturer.

The measurement of the dosage which results from the aerosol exposure is accomplished by use of deposition coupons (petri dishes) which are placed in the exposure chamber on the microcosm cart surface. Duplicate deposition coupons were used in nearly all exposures performed and these were intended to collect the total aerosol deposition on a known horizontal surface during the two-hour exposure period. The determination of the content of these coupons is discussed in the following section.

2.4.6 Phosphorus Analysis of Deposited Aerosol

An analytical method was required for quantifying phosphorus mass loadings on deposition coupons. The analytical method was required to be simple, rapid, and cost-effective. Base titration of an aqueous extract of the deposition coupons was considered as the most likely candidate for this analysis. Verification of the base titration method was made by comparison to the conventional colorimetric analysis of orthosphosphate.

Samples of deposition coupons were immersed in 50 mL of distilled water for a period of approximately 18 hours. A 5-mL aliquot of this extract was withdrawn for analysis of orthophosphate-phosphorus by the colorimetric procedure[27]. In this method, ammonium molybdate and potassium antimonyl

tartrate react in an acid medium with dilute solutions of phosphorus to form an antimony-phosphate-molybdate complex. This complex is reduced to an intensely blue colored complex by ascorbic acid. This color is analyzed at 880 μm using a spectrophotometer.

An evaluation of several samples was conducted to determine the presence of any polyphosphoric acids. This evaluation was performed by analyzing samples before and after addition of 5 N H_2SO_4. This procedure indicated no significant polyphosphoric acids were contained in the aerosol deposition samples. This was probably due to the long time period (>2 weeks) that coupons were stored before analysis. Ballou[1] has shown that the majority of reagent grade phosphorus pentoxide is hydroloyzed to orthophosphoric acid within 5 days at room temperatures. Thus, acid hydrolysis was not used for subsequent samples.

A second 5-mL aliquot of sample extract was withdrawn for analysis by titration. A calibrated pH electrode was immersed in the dissolved sample and titrated with an appropriate concentration of standard NaOH solution. For low exposures, a concentration of 0.01 N NaOH was used, while 0.1 N NaOH was used for more highly concentrated aerosol samples. Samples were titrated to a pH of 9.0 and the volume of titrant was recorded. The amount of P in mg contained in the deposition samples were calculated according to the equation below:

$$\text{mg P} = (EW)(N)(V)(DF)(GF)$$

where

EW = mg H_3PO_4/meq
= 49

N = Normality of titrant (meq/mL)

V = Volume of titrant (mL)

DF = Dilution factor

$$= \frac{25 \text{ mL}}{5 \text{ mL}}$$

$$= 5$$

GF = Gravimetric factor

$$= \frac{\text{At. Wt. P}}{\text{F.W. } H_3PO_4}$$

$$= \frac{31}{98}$$

$$= 0.32.$$

2.4.7 Microcosm Construction and Maintenance

The design, construction techniques, and maintenance of the terrestrial microcosms were similar to those used in other Battelle projects[13,28,29]. The basic design of the terrestrial microcosm was a 60-cm intact soil core encased in a 17-cm-diameter tube of Driscopipe® (Figure 2). Driscopipe® is an ultra-high molecular weight, high-density polyethylene pipe containing no plasticizers and is chemically inert. Microcosm tubes were cut from 40-foot lengths of Driscopipe® using a horizontal band saw and the ends were smoothed with a special facing machine rented from the Driscopipe® dealer. Each tube was acid-washed prior to further use.

Extraction of the 60-cm, intact soil cores required the use of a specially designed, steel driving-tube. The driving tube permitted soil cores to be taken with a backhoe in high clay content subsoil, such as the silt-loam soil used in this study from Battelle's West Jefferson facility. The microcosm tube fit inside the driving tube, which prevented warping and/or splitting of the microcosm tube due to the force required to push the encasement material into the ground.

Seventy intact soil cores were extracted from an undisturbed area of Battelle's West Jefferson complex on December 21, 1983. The cores were removed from the driving tube and were transported to the greenhouse. They were placed vertically on carts, the vegetation was removed, and RO water was added to prevent drying of the soil.

Twenty special moveable wooden carts were constructed to hold three intact soil core microcosms apiece (see Figure 1). The microcosms were placed in the carts on an acid-washed Buchner funnel (see Figure 2) covered by a thin layer of glass wool, and were leached with RO water. Sixty cores were selected from the seventy originally extracted, based on their leachability within a 24-hour period. These sixty cores were set in the Buchner funnels in the carts, the carts were packed with styrofoam insulation, the tops of the carts were anchored in place with wood screws, and the microcosm cores were sealed to the tops with DAP white sealant. Topsoil was collected from the same location where the microcosm cores were extracted and was used to top off the soil cores to uniform levels. All carts were labelled according to the experimental design outlined in Section 2.1.2.

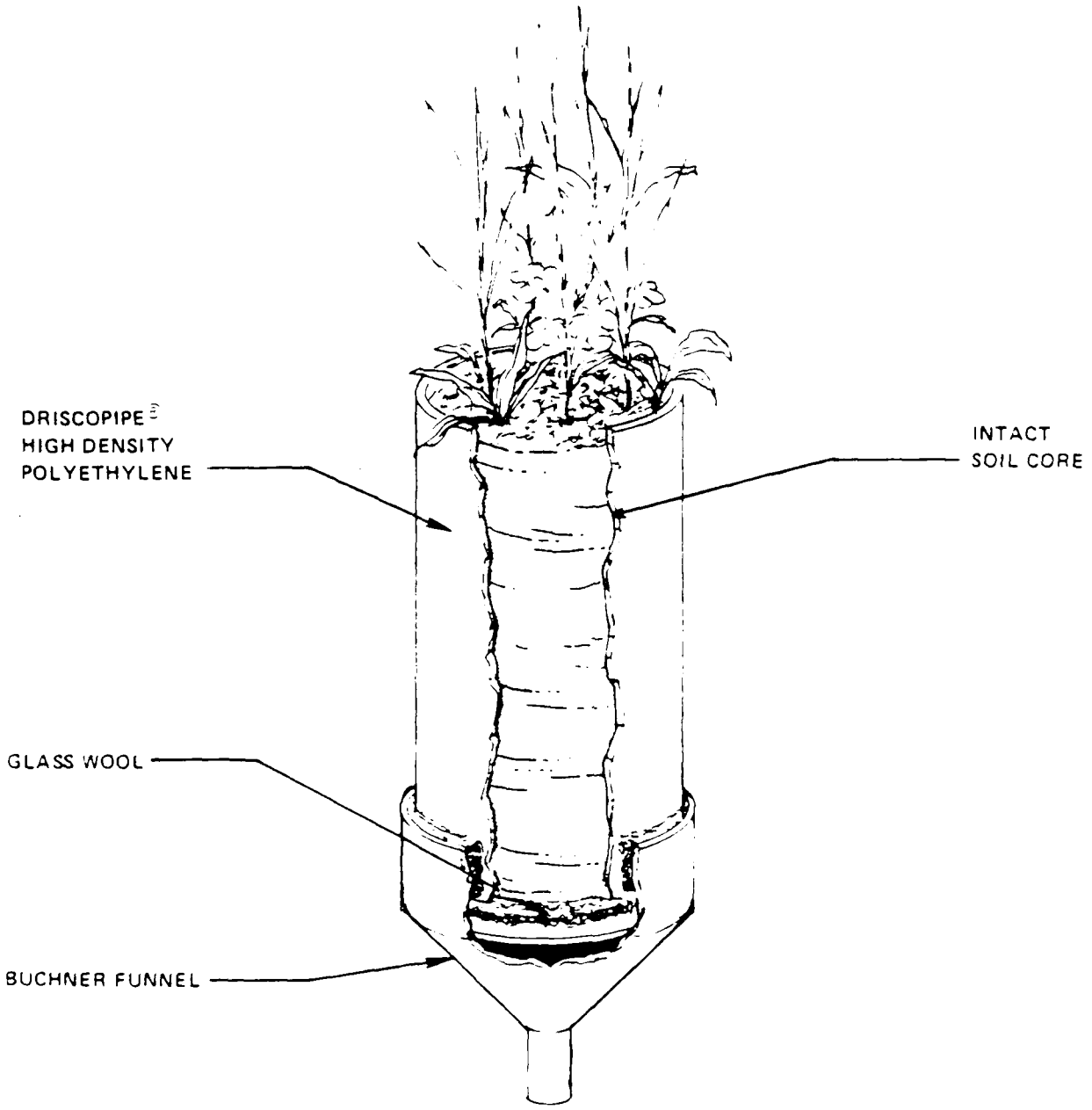


FIGURE 2. DESIGN OF THE TERRESTRIAL MICROCOSM

Microcosms were planted with the combined species used in earlier stress ethylene tests: perennial ryegrass, white sweetclover, and wheat. The seeding rate was two times greater than the rates recommended for the combined species in the Ohio Agronomy Guide(30). The microcosms were later thinned back or replanted as necessary to achieve the following number of viable plants per microcosm:

- perennial ryegrass - 9 plants
- white sweetclover - 5 plants, and
- wheat - 3 plants.

The resulting mixed-crop stand thickness after thinning was designed to be one-third of the individual species planting rate to avoid over-crowding. At the same time, the technique of over-planting and thinning back assured an identical species and stand thickness in each microcosm prior to smoke exposure.

Microcosms were distributed evenly between two greenhouse bays, 30 for RP/BR and 30 for WP/F. They were watered daily with 150 mL of half-strength Hoagland's nutrient solution until the nutrient stress determined from soil analysis (see Section 2.4.9) was eliminated. The quantity of Hoagland's solution used was calculated on the basis of fertilizer recommendations received from the Ohio Agricultural Research and Development Center, and the total mass loading of individual nutrient elements as a result of adding half-strength Hoagland's solution. Temperature and humidity were monitored daily and were controlled by supplemental humidification and manual and automatic initiation of heaters and coolers. A photoperiod of 16 hours light/8 hours dark was achieved by the use of metal halide lights, as necessary.

To avoid gradients of temperature, lighting, and humidity, as well as other potential space-related effects in the greenhouse, microcosms were rotated periodically around the greenhouse bays. The rotation occurred according to a specific plan to assure that each cart experienced similar overall growth conditions. Carts were labelled to indicate the type of the appropriate smoke to be applied, and also to assure that each cart always maintained its original compass direction during the rotation process. During

the first month after planting, carts were rotated twice a week. Thereafter, carts were rotated once each week.

During and after the exposure of microcosms to RP/BR and WP smokes, plants were monitored daily for signs of stress, such as discoloration, wilting, and leaching. After the nutrient deficiencies were corrected with Hoagland's solution, microcosms were watered with 150 mL of RO water on Monday and Wednesday of each week. In addition, Friday of each week of exposure, leaves of plants in microcosms were washed with 300 mL of simulated rainfall in order to mimic the washing of leaves that might be expected to occur in situ. The amount of water added to each microcosm was determined from published records of weekly precipitation for the Columbus, Ohio area. That is, the total precipitation for a normal growing season divided by the number of weeks during the growing period gave the average weekly rainfall. A spray nozzle attached to a hose was adjusted to mimic a reasonable drop size (to simulate rainfall), and was calibrated to deliver the desired amount of "rainfall" (RO water) in a specified time period. A special collar was devised that was placed over each microcosm during the watering process to assure that all the simulated rainfall impacted the microcosms. In addition to simulating the washing of leaves that would be expected to occur in situ, this procedure also served to transfer particulates to the soil, a process that would also occur naturally in the field.

All cores were monitored daily for insect infestation. During July of 1984 (shortly after the exposures to RP/F and WP were completed), some of the microcosms became infested with aphids. This problem was alleviated by spraying all microcosms with malathion [O,O-Dimethyl S-(1,2-dicarbethoxyethyl) phosphorodithioate]. A total volume of 1.9 L of spray containing 2.2 mL of active ingredient per liter were distributed evenly over all the microcosms using a hand sprayer.

2.4.8 Monitoring and Analysis of Microcosm Parameters

Several major parameters were monitored to detect effects of the obscurant smokes on the soil-plant system. These included analysis of plant nutrients in soil-core leachate, total above-ground plant biomass, and element uptake in above-ground plant biomass.

2.4.8.1 Leachate Collection and Nutrient Analysis. The design of the soil-core microcosm used in this study (see Figure 2) greatly facilitates the collection of soil leachate. This is important because several studies have shown that unstressed terrestrial ecosystems tend to conserve plant nutrients while stressed systems often lose important nutrients via leaching in soil water[31,32,33]. Thus, the relative physical or chemical stress placed on a terrestrial ecosystem may be indicated by the degree of loss of soluble nutrients in soil leachates.

Soil leachates were collected for analysis on three different dates in 1984: May 15, June 18, and July 23. To obtain leachate, RO water was added to the top of each soil-core microcosm and allowed to percolate through the core and Buchner funnel, into the acid-washed collection flask under the funnel. The leachate volume was recorded, leachates from the three microcosms in a cart were pooled, and the pooled leachate was filtered through a 0.45-micron filter and stored at 4°C in the dark.

Within 24 hours after the first leachates were collected, they were analyzed for ammonium-nitrogen (NH_4-N) via a specific ion electrode. In all cases the concentration of NH_4-N was similar to that in distilled water. These leachates also were analyzed for total organic carbon (TOC) using an Oceanography Institute Model 524 Carbon Analyzer. It was assumed that the TOC values were equal to dissolved organic carbon (DOC), since samples were filtered through a 0.45-micron filter. Nitrate-nitrogen (NO_3-N) was determined using the automated Cd reduction method with an atomic absorption spectrophotometer (AAS). Calcium (Ca) and potassium (K) were determined using a Model 303 Perkin-Elmer AAS.

Leachates from the second and third leachings were treated similarly except for nutrient analyses. Based on the results from the first leaching, subsequent leachates were only monitored for NO_3-N and Ca.

2.4.8.2 Biomass Harvest and Weighing. Plants in microcosms were harvested on May 18 and July 26, which is 11 and 81 days, respectively, after microcosms were first exposed to the phosphorus smokes. Crops were cut at about 5 cm above the soil surface and the biomass was oven-dried at 100°C for 72 hours. In the first harvest, all plant species in a microcosm were harvested, dried, and weighed together. In the second harvest, however, each of the three species was separately dried and weighed from each microcosm.

2.4.8.3 Element Uptake--Sample Preparation and Analysis. Plant biomass from each of the two harvests was ground in a Wiley mill, mixed, subsampled, digested with perchloric and nitric acids and analyzed for selected elements. Biomass from all species in each of the three microcosms in a given cart (single treatment level) was combined prior to grinding. Biomass from the first harvest was analyzed for 23 elements (Al, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Mo, Na, Ni, P, Pb, Se, Sr, Ti, Tl, V, Y, Zr) by inductively coupled-argon-plasma spectroscopy (ICAP), and for As and Se by hydride generation atomic absorption spectrophotometry (AAS). Based on statistical analysis of element uptake data from the first harvest, the biomass from the second harvest was analyzed for 5 elements (Al, Cr, Mo, P, and Pb) using ICAP and for As by hydride generation AAS.

2.4.9 Supplementary Studies

In order to assist in the interpretation of biomass data, two types of data were collected that were peripheral to the main study design. Measurements were made on leaf surface area of each species and on incident light to plants during exposures.

2.4.9.1 Leaf Surface Area. Immediately after the second harvest, the total leaf area of each species in each microcosm was determined using a Lambda LI 3000 portable area meter. This value was considered important, because one of the primary modes of smoke impact on plants was believed to be aerosol desposition on the leaves.

2.4.9.2 Light Intensity During Exposure. Light intensity was measured before and during smoke exposures by a quantum sensor supported at plant height near the center of the three microcosms. Measurements were recorded in micro-einsteins per square meter per second using a Quantum/Radiometer/Photometer Meter, Model LI-185A made by Lambda Instruments Corporation. Measurements were made with mercury vapor lights off and on before igniting the smoke material and at 0, 1, 5, 10, 20, 30, 60, 90, and 120 minutes after ignition. Since only one meter was used, measurements were only made for one exposure level of one smoke during a given 2-hour exposure period.

2.4.10 Soil Characterization

Prior to extracting microcosms from the field area, soil samples from the 0 to 15-cm depth and from the 15 to 61-cm depth were taken. These were analyzed by the Soil Analysis Division of the Research-Extension Analytical Lab, The Ohio Agricultural Research and Development Center, Wooster, Ohio. Fertilizer recommendations were included in the analysis and were considered when adding nutrient solution to microcosms as described in Section 2.4.7.

Soils from microcosms exposed to various levels of RP/BR or WP smokes over an eight-week period were characterized for pH, electrical conductivity (EC) and cation exchange capacity (CEC). pH is a measure of the hydrogen ion concentration in the soil solution; EC is a measure of soluble salts; and CEC is an indicator of the ability of the soil to retain important nutrient cations, such as calcium, magnesium, and potassium.

Approximately four weeks after the last exposure, soil from the top 15 cm of each replicate microcosm in a cart was pooled, air-dried, and screened through a 2-mm-mesh sieve. pH was determined with a calibrated and standardized Orion model 601A digital ion analyzer using a 1:1 soil-to-distilled water mixture, according to the method of McLean[34]. The EC of each pooled soil was determined according to the method of Watson[35] by using a Yellow Springs Instrument (YSI) model 32 digital conductivity meter with a YSI model 3401 conducting cell. A 1:2 soil-to-deionized water mixture was used for EC measurements.

Cation exchange capacity of pooled soils was determined according to Allen et al.[36]. Sieved soil was equilibrated for 1 hour with 1 M ammonium acetate and the excess ammonium was removed by washing with ethanol. The sorbed ammonium ions, which saturate the cation exchange sites, were displaced with 5 percent potassium chloride solution. Displaced ammonium was quantitatively determined using an ammonium ion-specific electrode. Cation exchange capacity was then calculated based on the proportionality of displaced ammonium ions to cation exchange sites and taking into consideration all dilutions, equivalent weights, and soil moisture content.

2.5 Soil Respiration Study

Potential effects of RP/BR and WP smokes on soil microorganisms were investigated using a soil respiration technique. This procedure was similar to another study by Arthur et. al.[18]. The soil respiration test measures the metabolic potential of a diverse group of soil heterotrophic microorganisms instead of one or a few indicator species. This test has been shown to be an indicator of toxic effects to soil microbial communities and compared well with results obtained from higher plant studies[12,18].

To examine the effects of the obscurant smokes on soil microbial respiration, approximately 300 g of soil were removed from the top 15 cm of each microcosm that had been previously exposed to RP/BR or WP smoke at target doses of 0, 100, 300, 600, or 1500 mg/m^3 for eight weeks (see Section 2.2.4). Each soil sample was mixed thoroughly and screened through a 2-mm-mesh stainless steel sieve. Portions of sieved soil (150 g each) from the three replicate microcosms representative of each treatment were combined and mixed thoroughly. From this 450-g sample, triplicate subsamples were oven-dried (110°C) overnight, cooled in a dessicator, and weighed for moisture determination. The bulk of the soil was maintained at field-moist conditions (air-drying was avoided).

One-hundred grams (on an oven-dry weight basis) of sieved, field-moist soil, representative of each experimental treatment, were distributed into each of triplicate acid-washed 0.95-l glass jars. One gram of finely ground alfalfa meal was mixed with the soil in each jar to provide an environmentally relevant microbial substrate with a narrow carbon-to-nitrogen ratio. Enough distilled water to reach a total moisture content of 70 percent field capacity was added to each jar. An alkali trap, containing 10.0 mL of 0.6 N NaOH, was added to each jar to trap evolved CO_2. The alkali was contained in a wide-mouth nalgene container that rested on a specially devised platform to keep the alkali trap off of the soil surface. Jars were incubated in the dark at 23°C, and jars were weighed periodically to monitor for soil moisture loss. Triplicate blanks (no soil) were also included.

During the first week of the experiment, the alkali traps were changed daily for four days. For the next three weeks, traps were changed three times per week, and two times during the last week of the experiment. To change a trap, the used alkali was removed, 5.0 mL of 1.3 N $BaCl_2 \cdot 2H_2O$

were added to precipitate absorbed CO_2 as $BaCO_3$, 10.0 ml of fresh 0.6 N NaOH were added to the traps, and the jars were returned to the incubator. The precipitated alkali was titrated to pH 9.0 with tris-standardized 0.6 N HCl using a Fisher autotitrator. Blanks were treated similarly. Section 2.6.5 describes the statistical analysis of the soil respiration data, which are presented as cummulative milligrams of carbon dioxide-carbon (CO_2-C) evolved over time.

2.6 Statistical Methods

The following descriptions apply to both RP/BR and WP smokes. The exposures for each smoke were designed and carried out as two separate studies and were analyzed as such. Data from the preliminary stress ethylene tests, the range-finding tests and the soil respiration study were subjected to statistical analysis in order to detect statistically significant effects of smoke exposure. The majority of these analyses were done using the Statistical Analysis system (SAS) computer package[37]. The data were first plotted to identify possible outliers and to reveal general trends. Summary tables of means and standard deviations for each treatment group were generated. One- and two-way analysis of variance (ANOVA) were used to detect differences between treatment groups[38]. Linear and quadratic regression curves were used to describe the relationship between exposure and the measured responses. The formulae for these curves are $y = b_0+b_1x$ and $y = b_0+b_1x+b_2x^2$, respectively, where y is the response and x is a measure of exposure[39]. Standard methods of regression analysis were used to test whether the fitted curves were significantly better descriptors of the data than a horizontal (no response) line. A significance level of 0.05 was used throughout. If the p-value for a particular test is less than 0.05 then the null hypothesis (no effect) is rejected and the treatment effect is judged to be statistically significant.

Measurements from individual microcosms located on the same cart, and hence exposed in the same exposure chamber, were never regarded as independent replicates (see Section 2.1.2). In some cases (e.g., leachates) a single measurement was obtained from a pooled sample. In other cases when individual measurements were available (e.g., biomass) an average was taken and this value was used in the statistical analysis. Thus, in all cases involving exposure chambers the microcosm group (or cart) was the unit of replication and not the individual microcosm.

2.6.1 Stress Ethylene Data

Ethylene concentration (ppb) was regressed against dose for each plant species. The dose was expressed as grams of RP/BR burned or as the number of pellets of WP/F burned. A linear regression line was fitted and estimates of the slope, intercept and correlation coefficient were obtained. These results were used to determine exposure levels and modify the design of the range finding experiments.

2.6.2 Nutrient Loss Data

Three leachate collections were made. Because leachate from each group of three microcosms was pooled, each data point represents the combined response of three microcosms. At the first leachate collection TOC, K, NO_3-N and Ca were measured and analyzed. In the first leachate, the nutrient NH_4-N was also chemically analyzed, but not statistically analyzed, because all values were below detection. At the second and third leachate collections only NO_3-N and Ca were analyzed. Values that were less than the detection limit for that nutrient were set equal to the detection limit for the purpose of analysis. Two-way ANOVA (cart x dose) and linear regression (with target level as the independent variable) were used to detect treatment affects.

For NO_3-N and Ca a cumulative nutrient loss was calculated by summing the individual nutrient losses over the three collection periods. This cumulative loss was analyzed by two-way ANOVA and dose responses were described by quadratic or linear regressions. A quadratic curve was used if the quadratic term of the regression was statistically significant ($p < 0.05$). Otherwise a linear curve was used. Since the cumulative nutrient loss spanned the entire exposure period, total phosphorus deposition (ug/cm^2) was used as the independent variable. This variable is a more accurate estimate of actual exposure over the whole experiment than the target level (see Section 3.3.1).

2.6.3 Biomass Data

Biomass data were individually and cumulatively analyzed from two harvests. At the first harvest, total biomass (g) was measured. At the

second harvest, the dry weights of wheat, sweetclover and ryegrass were measured and analyzed separately. Total biomass for the second harvest and for the entire experiment were calculated and analyzed. Biomass measurements were averaged over each group of three microcosms to give a single value per cart for each exposure level. Two-way ANOVA was used to test for cart and exposure effects. For the first harvest, linear regression was based on target exposure levels. For the second harvest, phosphorus deposition was used as the dependent variable. Preliminary plots indicated a non-linear dose response in some cases. This was handled by fitting a quadratic regression curve. If the quadratic term was not statistically significant then a linear curve was fitted instead.

2.6.4 Element Uptake Data

Two sets of chemical analyses were done on element concentrations in plant tissue from each of the two harvests. The first analysis measured twenty-four elements (see Section 2.4.8.3). Based on which elements had significant or near significant results in the first analysis, six elements were selected and analyzed in the second analysis. For each element in the first analysis, a two-way ANOVA was used to test for cart and target smoke exposure level effects, and a linear regression was fitted using target level as the dependent variable.

The same approach was used for the six elements measured in the second set of chemical analyses with the exception that phosphorus deposition was the dependent variable in the regression analysis. Plots of the data suggested that the quadratic model $y = b_0+b_2x^2$ (where y = arsenic concentration, and x = P deposition) was more appropriate than a linear model for arsenic. Therefore, this quadratic model was fitted to the arsenic data but not to the other element data. Note that this quadratic model is different from the quadratic models ($y = b_0+b_1x+b_2x^2$) fitted in the leachate and biomass analyses. This is because the scatterplot suggested a quadratic trend where the minimum level of arsenic concentration in plant tissue occurred when the deposition of phosphorus level was zero, thus the model $y = b_0+b_2x^2$ appeared more appropriate than $y = b_0+b_1x+b_2x^2$. This was confirmed statistically by comparing the fit of the two models.

2.6.5 Soil Respiration Data

Soil respiration data were analyzed to determine the effect of the target smoke level on the rate of decline of CO_2-C (in mg) over time. Steps in the analysis involved calculating daily and cumulative CO_2-C values, making scatter-plots of daily and cumulative CO_2-C versus day, selecting a model which best described the relationship between CO_2-C and time (in days) for each cart, and using a one-way ANCVA and linear regression to test the rate of decline of CO_2-C soil respiration over time (b-parameter in the selected model for each cart).

Cumulative CO_2-C was calculated as follows:

$$CO_2\text{-}C = \sum_{i=1}^{j} [(B-V)NE]_i$$

where

V = daily average (averaged over 3 microcosms per cart) mL of acid for end-point titration of the alkali in the CO_2 traps from treated soils,

B = daily average (averaged over 3 microcosms per cart) mL of acid for end-point titration of the alkali in the CO_2 traps from method blanks,

N = normality of acid (0.6569 eq/mL),

E = equivalent weight of CO_2-C, i.e., 6 mg/meq, and

j = number of titrations (15 for each smoke).

For each group of three microcosms the daily CO_2-C evolution was plotted against time (days) to reveal any trends and to suggest models for describing the relationship between CO_2-C and time. Four models were considered:

1) $y = ab^t$

2) $y = ae^{bt}$

3) $y = a+b\log_e t$

4) $y = a+bt$

where y is CO_2-C and t is time in days. A best-fitting model was selected on the basis of low p-values and high R^2 (coefficient of determination) values. R^2 is the proportion of the total amount of variation in the data that is explained by the fitted model(39).

Using the rate of decline (b parameter) for each microcosm group, a one-way ANOVA was used to test for the effect of target smoke exposure levels on the rate of decline. A scatterplot of the rate of decline versus target smoke exposure suggested a quadratic relationship so a quadratic model, $b = a_0+a_1x+a_2x^2$ was fitted, where b is the rate of decline, and x is the target smoke exposure. When the quadratic term was not statistically significant then a linear curve was fitted instead.

2.6.6 Supplementary Data

Two types of supplementary data were also analyzed: leaf surface areas (in cm^2) of wheat, sweetclover, and ryegrass and light intensity during exposure (microeinsteins/m^2/sec). The leaf surface areas were measured along with the dry weights at the second biomass harvest.

2.6.6.1 Leaf Surface Areas. The mean surface areas of wheat, sweetclover, and ryegrass for each cart (averaged over microcosms per cart), at each target smoke exposure level were analyzed. A two-way ANOVA was used to test for cart and smoke exposure effects. Plots of mean surface areas for wheat, ryegrass, and sweetclover versus phosphorus deposition were made. Since the plots suggested a possible quadratic trend in the data, a quadratic model, $y = b_0+b_1+b_2x^2$ (where y is the mean surface area, x is the deposition of phosphorus level), was fitted to the data. When the quadratic term was not significant ($p > 0.05$), a linear model, $y = b_0+b_1x$, was fitted.

2.6.6.2 Light Intensity During Exposure. The light intensity at certain time intervals (1 minute, 5 minutes, 10 minutes) after time zero was divided by the light intensity at time zero. Plots of light intensity ratio versus the peak phosphorus level for a given day were made. The plot for RP/ER smoke suggested a linear model: $y = b_0+b_1x$ (where y is the light

intensity ratio, and x is the peak phosphorus level). The plots for WP smoke suggested a hyperbolic model, $y = b_0+b_1(\frac{1}{x})$ (where x and y are the same as before).

3.0 RESULTS AND DISCUSSION

The results of this project are presented and discussed according to major topic areas: preliminary tests, soil leachate characterization, microcosm tests, and soil respiration experiment. Each major topic is broken down further as necessary in the following sections.

3.1 Preliminary Tests

The two major preliminary tests that were conducted prior to the microcosm exposures were exposure characterization and plant stress-ethylene testing. The exposure characterization was necessary to determine the combustion techniques and smoke material quantities appropriate for the small stress-ethylene test chamber. The results of the stress-ethylene tests were used to determine appropriate exposure levels for the microcosm tests.

3.1.1 Exposure Characterization in the Stress-Ethylene Chamber

Test conditions measured in the small stress-ethylene chambers included temperature and relative humidity, as well as mass concentration of the aerosol particles suspended in the chambers. Results of the temperature readings taken during an exposure indicated no thermal effects due to the smoke combustion. Measurements of the relative humidity indicated that the chamber atmosphere approached saturation within about 40 minutes of the insertion of the plants. This resulted in changes in the watering protocol prior to the microcosm tests to reduce the relative humidity to more typical levels.

Preliminary tests performed with both smokes indicated no increase in ethylene production in treated versus control plants from exposures up to several hundred mg/m^3. To increase the total exposure of the plants in a short-duration (2-hour) test, multiple periods of aerosol generation were used, as discussed in Section 2.2. The measured aerosol concentrations are presented in Figures A-1 and A-2 in Appendix A. These figures illustrate the rapid removal of aerosol mass due to settling in the chambers, which was confirmed by visual observations of the plant surfaces. These aerosol

concentrations do not provide a good measurement of the actual dose applied, since that is given by the mass of settled material, but the minimum concentration required to produce a plant stress-ethylene response (roughly 20,000 mg/m^3) provided guidance in selecting the target concentrations to be employed in the microcosm tests discussed below.

3.1.2 Plant Stress Ethylene Response

The plant stress-ethylene test was conducted to determine the acute response (increased ethylene release) of each of the three plant species planned for use in the microcosm test. The end-points (mean ethylene concentrations) from this test are presented in Tables 1 and 2. These results were interpreted two ways. First, the lowest smoke dose resulting in a mean ethylene concentration above the stress-response threshold value for any one of the three plant species was determined. The stress-response threshold value is considered to be 1.5 times the mean ethylene concentration of the room-air-control plants[22]. This acute test threshold was used to set the exposure levels for the chronic microcosm test. Second, dose-response curves were plotted for smoke dose versus mean ethylene concentration (Figures 3 and 4). The four doses of each smoke represent 1, 2, 3, or 4 repeat combustion events (within a 2-hour period), corresponding to aerosol concentrations of approximately 20,000 mg/m^3 for WP/F and 18,000 mg/m^3 for RP/BR for each event. Each figure includes separate dose-response curves for each of the three species of plants. A linear regression analysis was performed in each case and estimates of the slope, intercept, and correlation coefficient are shown on Figures 3 and 4. The slopes of the dose-response curves were used to compare the smoke sensitivity between species.

White sweetclover appeared to be more sensitive than the other two species, in that only one combustion event of either smoke (18,000 to 20,000 mg/m^3) resulted in a mean ethylene concentration that exceeded the stress-response threshold value (Table 1). With both smokes, the concentrations required to exceed the stress-response threshold value were considerably higher than realistic field exposures (50 to 400 mg/m^3). Thus, the doses selected (see Section 2.4.1) for the chronic, repeated exposure of microcosms were below the smoke levels (18,000 to 20,000 mg/m^3) required to elicit an

TABLE 1. STRESS ETHYLENE EVOLVED FROM WHITE SWEETCLOVER, PERENNIAL RYEGRASS, AND WHEAT WHEN EXPOSED TO VARIOUS CONCENTRATIONS OF COMBUSTED RP/BR (n = 3)

RP/BR Treatment Otherwise Indicated	Mean Ethylene Concentrations (ppb ± SD)		
	White Sweetclover	Perennial Ryegrass	Wheat
Room Air	12.3 ± 0.81	7.25 ± 2.75	28.5 ± 3.3
5 g (1 x 5 g)(a)	43.0 ± 10.6	9.6 ± 2.0	18.4 ± 3.7
10 g (2 x 5 g)	106.5 ± 14.4	25.0 ± 9.2	19.3 ± 3.3
15 g (3 x 5 g)	252.5 ± 102.4	92.5 ± 18.7	29.4 ± 10.0
20 g (4 x 5 g)	174.5 ± 17.8	133.7 ± 44.1	71.4 ± 10.6
Positive Control (0.5 ml Cl)	20.2 ± 5.9	5.7 ± 0.75	28.5 ± 8.6

(a) Values in parentheses indicate number of times the indicated weight of RP/BR was combusted in the chamber. Each combustion resulted in a peak aerosol concentration of roughly 18,000 mg/m^3.

TABLE 2. STRESS ETHYLENE EVOLVED FROM WHITE SWEETCLOVER, PERENNIAL RYEGRASS, AND WHEAT WHEN EXPOSED TO VARIOUS CONCENTRATIONS OF COMBUSTED WP/F (n = 3)

RP/BR Treatment Otherwise Indicated	Mean Ethylene Concentrations (ppb ± SD)		
	White Sweetclover	Perennial Ryegrass	Wheat
Room Air	17.0 ± 1.5	14.8 ± 3.3	21.0 ± 4.9
2 pellets (1 x 2)(a)	33.0 ± 6.4	22.2 ± 4.4	13.9 ± 1.5
6 pellets (2 x 3)	66.2 ± 3.2	48.2 ± 12.0	25.5 ± 1.2
9 pellets (3 x 3)	153 ± 38.6	84.0 ± 8.8	62.7 ± 10.7
12 pellets (4 x 3)	202 ± 112	56.4 ± 2.6	51.0 ± 3.2
Positive Control (0.5 ml Cl)	96 ± 83 (n = 2)	11.3 ± 4.0	26.5 ± 2.1 (n = 2)

(a) Values in parentheses indicate number of times the indicated number of WP/F pellets were combusted in a chamber. Each combustion resulted in a peak aerosol concentration of roughly 20,000 mg/m^3.

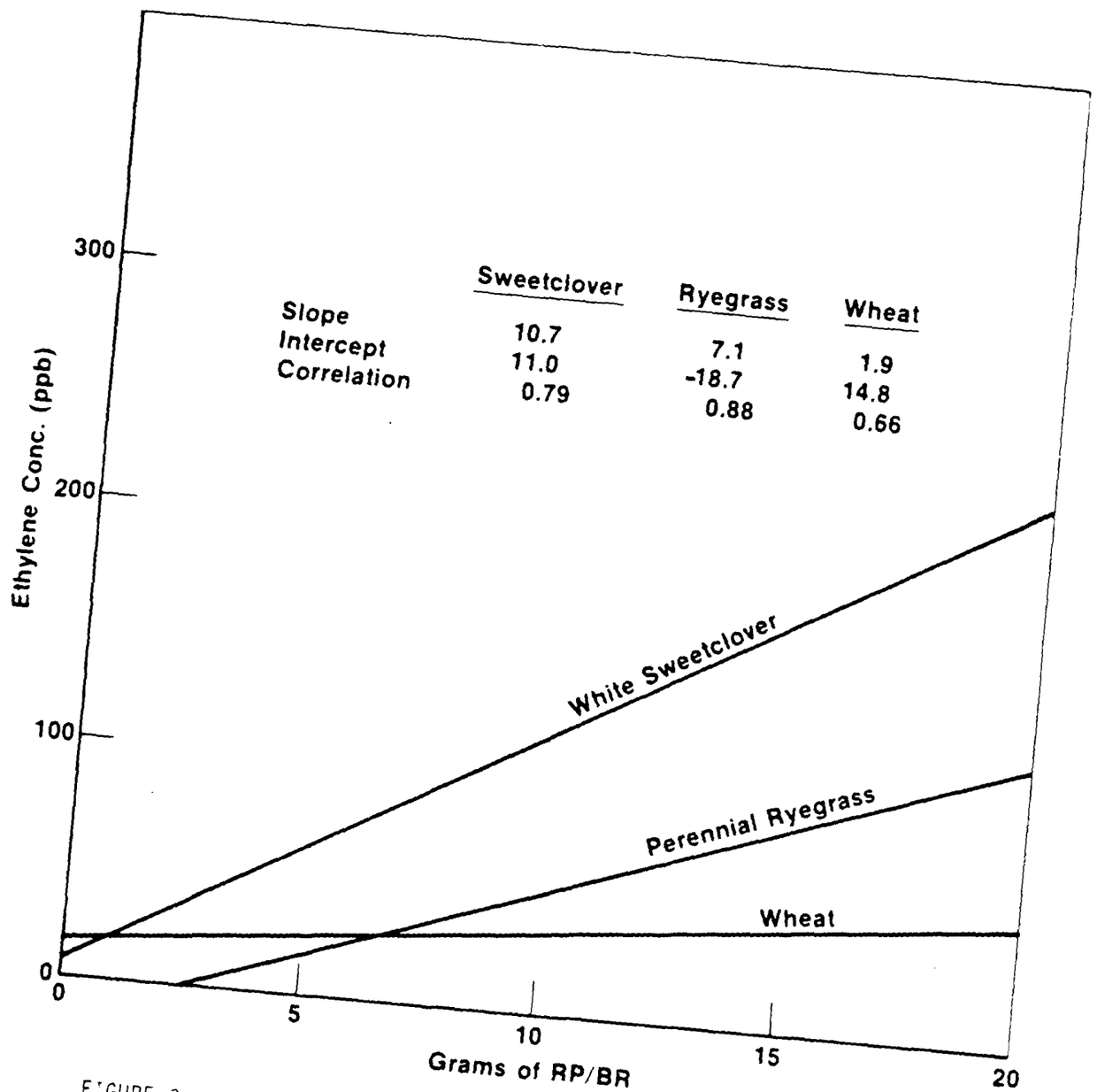


FIGURE 3. LINEAR REGRESSION ANALYSIS OF STRESS ETHYLENE PRODUCTION FROM THREE SPECIES OF PLANTS VERSUS GRAMS OF RP/BR COMBUSTED IN THE STRESS-ETHYLENE CHAMBERS

[Each repeat combustion (5 g of RP/BR) resulted in an aerosol concentration of roughly 18,000 mg.m3]

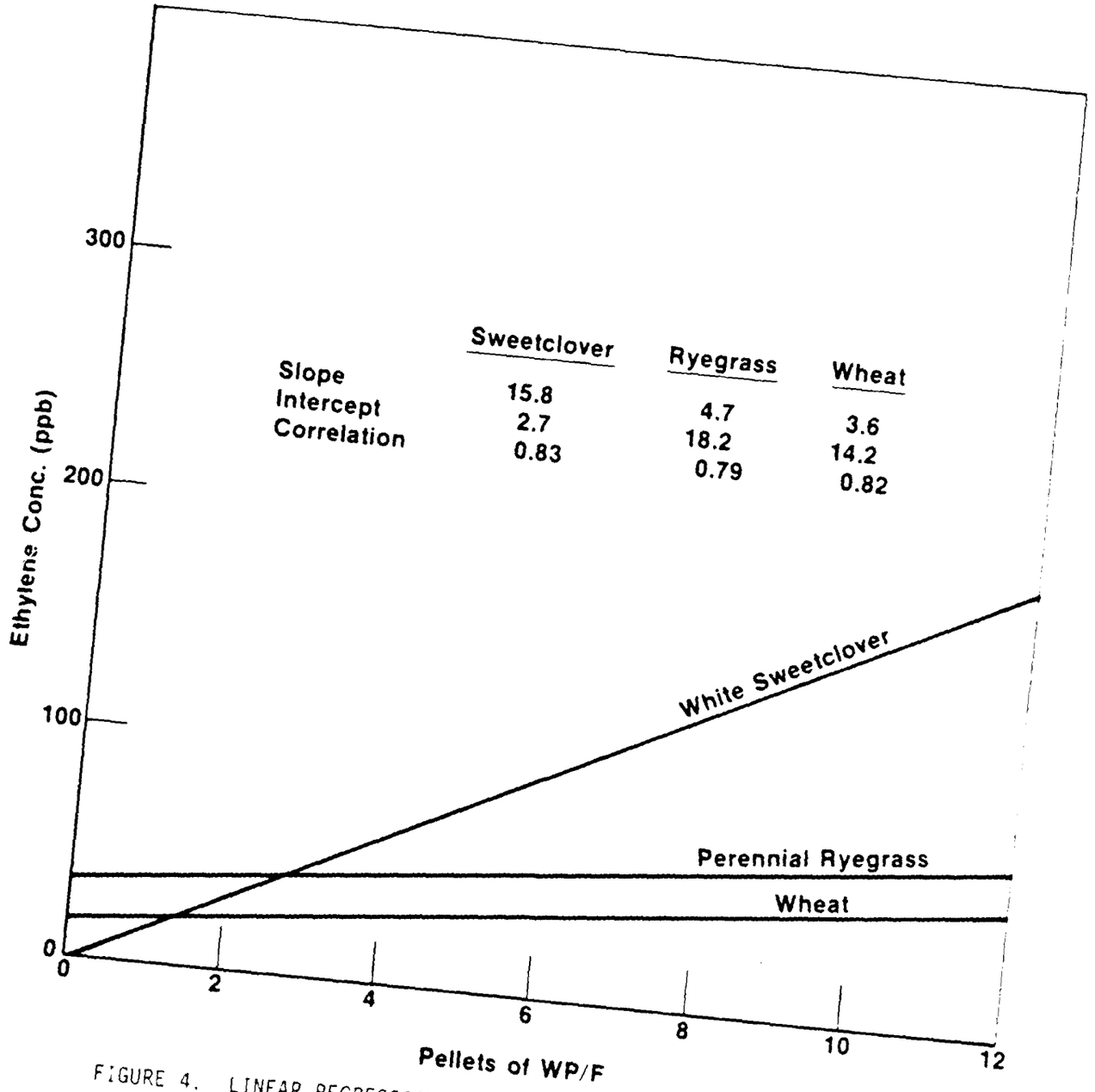


FIGURE 4. LINEAR REGRESSION ANALYSIS OF STRESS ETHYLENE PRODUCTION BY THREE SPECIES OF PLANTS VERSUS THE NUMBER OF PELLETS OF WP/F COMBUSTED IN THE STRESS ETHYLENE CHAMBERS

[Each repeat combustion (2-3 pellets of WP/F) resulted in an aerosol concentration of roughly 20,000 mg/m^3]

acute, threshold ethylene response from the most sensitive plant species, sweetclover, due to exposure to either phosphorus smoke.

For both smokes with all three plant species, the slopes of the regression line of dose versus ethylene production were positive and significantly different from zero (see Figures 3 and 4). The magnitude of the slopes for sweetclover illustrates further the relative sensitivity of this species to high doses of the smokes, compared to wheat and perennial ryegrass. In addition, the order of smoke sensitivity for either smoke from most to least sensitive is sweetclover, ryegrass, and wheat.

The original study plan had assumed that much lower concentrations of the obscurant smokes would elicit plant stress ethylene production above the stress-response threshold. It was also assumed that higher doses of smokes would result in plant lethality, from which an EC_{90} (concentration eliciting lethality in 90 percent of plants tested for some duration) was to have been calculated. These data were to have served as upper and lower boundaries of exposures for the subsequent microcosm tests. As it turned out, no plant lethality was noted, even at the highest of RP/BR or WP/F smoke concentrations tested.

The results of these preliminary tests illustrate that a relatively simple screening test, such as stress ethylene, can be used to design effective larger-scale experiments. If the stress-ethylene preliminary test had not been done, microcosms would have been exposed initially to a range of smoke concentrations much broader than that finally used. This would have been considerably more expensive than using the stress ethylene test to refine the subsequent microcosm exposures.

Use of the stress ethylene technique as a screening test is also supported by results from the microcosm exposures. The stress ethylene test predicted little or no toxicity due to exposure to very high concentrations of RP/BR or WP/F smokes, and the soil-core microcosm results confirmed that both smokes are relatively innocuous to the terrestrial environment. These results are discussed in detail in Section 3.3.

3.2 Soil Leachate Characterization

Nutrient analysis of the soil leachate is presented in Table 3 for K, Ca, and NO_3-N. Statistical analysis of the nutrient levels indicated that none of the treatment levels of applied smoke aerosol was significantly different from the controls. Ammonia-nitrogen was below detection levels in all leachate samples. Total organic carbon was analyzed for a single leachate sample from each leachate fraction and the 20 g/m^3 aerosol treatment level. These results are presented in Table 4. While the TOC cannot be analyzed statistically due to the lack of replication, TOC levels in the treated soil leachate are not greatly different than the controls.

These data indicate that the buffering capacity represented by an approximate soil depth of 15 cm has not been affected by the deposition of phosphate aerosols. Physicochemical effects of deposited aerosols in the microcosm soil would not be expected to be based on acidity levels associated with the aerosols at the highest treatment level. Thus, any leaching loss of nutrients from the microcosms associated with aerosol deposition may be attributable to biologically mediated processes.

3.3 Microcosm Tests

Results from the preliminary tests and soil leachate characterization were used to determine, respectively, the smoke exposure levels and nutrients analyzed in leachate for the microcosm tests. The results from the microcosm tests described below include data evaluation from exposure characterization, nutrient loss, biomass yield, element uptake by plants, and soil respiration studies.

Statistical analyses of ecosystem parameters included regression analysis (linear or quadratic) to determine significant trends, ANOVA to determine if there were significant differences between doses, and Bonferroni's test to determine significant differences between specific doses and the controls (see Section 2.6). If a trend existed based on regression analysis, it was considered important whether or not dose differences were detected by ANOVA or Bonferroni's test. An attempt was made to explain all trends from a biological point of view.

TABLE 3. SELECTED NUTRIENT ANALYSIS OF SOIL LEACHATE

Smoke	Fraction	Dose for Deposited Aerosol (mg/m^3)	K (mg/L) Mean	± SD(a)	Ca (mg/L) Mean	± SD(a)	NO_3-N (mg/L) Mean	± SD(a)
RP/BR	1	Control	2.70	(0.08)	18.25	(4.60)	0.69	(0.88)
		100	2.59	(0.39)	15.00	(2.83)	0.64	(0.76)
		1500	2.81	(0.23)	16.25	(1.77)	0.83	(1.07)
		20,000	2.31	(0.16)	14.75	(8.13)	0.37	(0.37)
	2	Control	1.71	(0.39)	3.50	(0.00)	0.42	(0.23)
		100	1.76	(0.16)	3.50	(1.41)	0.41	(0.55)
		1500	1.98	(0.00)	3.50	(0.00)	0.51	(0.45)
		20,000	1.71	(0.39)	3.55	(1.41)	0.41	(0.05)
	3	Control	1.76	(0.31)	3.50	(0.00)	0.38	(0.04)
		100	1.49	(0.39)	4.50	(1.41)	0.45	(0.04)
		1500	1.82	(0.08)	6.50	(2.83)	0.47	(0.34)
		20,000	1.76	(0.00)	3.50	(0.00)	0.36	(0.06)
WP	1	Control	2.70	(0.08)	18.25	(4.60)	0.69	(0.88)
		100	2.42	(0.16)	20.75	(3.89)	0.21	(0.17)
		1500	2.53	(0.00)	14.00	(5.66)	0.76	(0.91)
		20,000	2.42	(0.31)	12.50	(4.95)	0.62	(0.53)
	2	Control	1.71	(0.39)	3.50	(0.00)	0.42	(0.23)
		100	1.32	(0.00)	3.00	(0.71)	0.31	(0.04)
		1500	1.93	(0.23)	4.00	(0.71)	0.49	(0.31)
		20,000	1.87	(0.31)	3.75	(0.35)	0.36	(0.00)
	3	Control	1.76	(0.31)	3.50	(0.00)	0.38	(0.04)
		100	1.27	(0.08)	1.50	(0.00)	0.34	(0.05)
		1500	1.65	(0.16)	3.75	(1.77)	0.51	(0.16)
		20,000	1.76	(0.46)	3.75	(1.06)	0.26	(0.04)

(a) Standard deviation (n = 2).

TABLE 4. TOC ANALYSIS OF SOIL LEACHATE

Fraction	Treatment	TOC, ppm
1	Control	36.7
	RP/BR	40.0
	WP	45.4
2	Control	13.6
	RP/BR	13.7
	WP	14.1
3	Control	9.7
	RP/BR	11.3
	WP	10.3

3.3.1 Exposure Characterization

Several variables were measured in each chamber during the tests to characterize the microcosm exposures. Temperature and relative humidity were recorded at prescribed times during the exposures and the average values for the RP/BR and WP smoke exposures are presented, respectively, in Appendix A, Tables A-1 and A-2. During the two-hour exposures, the temperature within the chamber typically rose about 5°C due to the lighting employed during the exposures. It should be noted that the control chamber was also lighted so that all microcosms were subject to this influence. The relative humidity in the chambers also typically rose due to transpiration of the plants. The watering schedule of the plants was chosen to minimize the RH increase during the exposures, which was usually only about 10-15 percent increase.

The initial aerosol concentration in the chambers was measured to provide a check on the consistency of the smoke generation, and to provide a measure which can be related to field experience. This information is presented below.

The mass of aerosol deposited was determined using deposition coupons, which act as receptors in the same mode as the microcosms. That is, the settling of smoke particles on the surfaces in the microcosms is the mode by which the relevant dosage is applied. So even if the supply of fresh smoke is maintained at a constant level in the chambers, and this airborne concentration is measured, this measurement does not describe accurately the applied dosage. The measurements of deposited phosphorus are presented in Section 3.3.1.3.

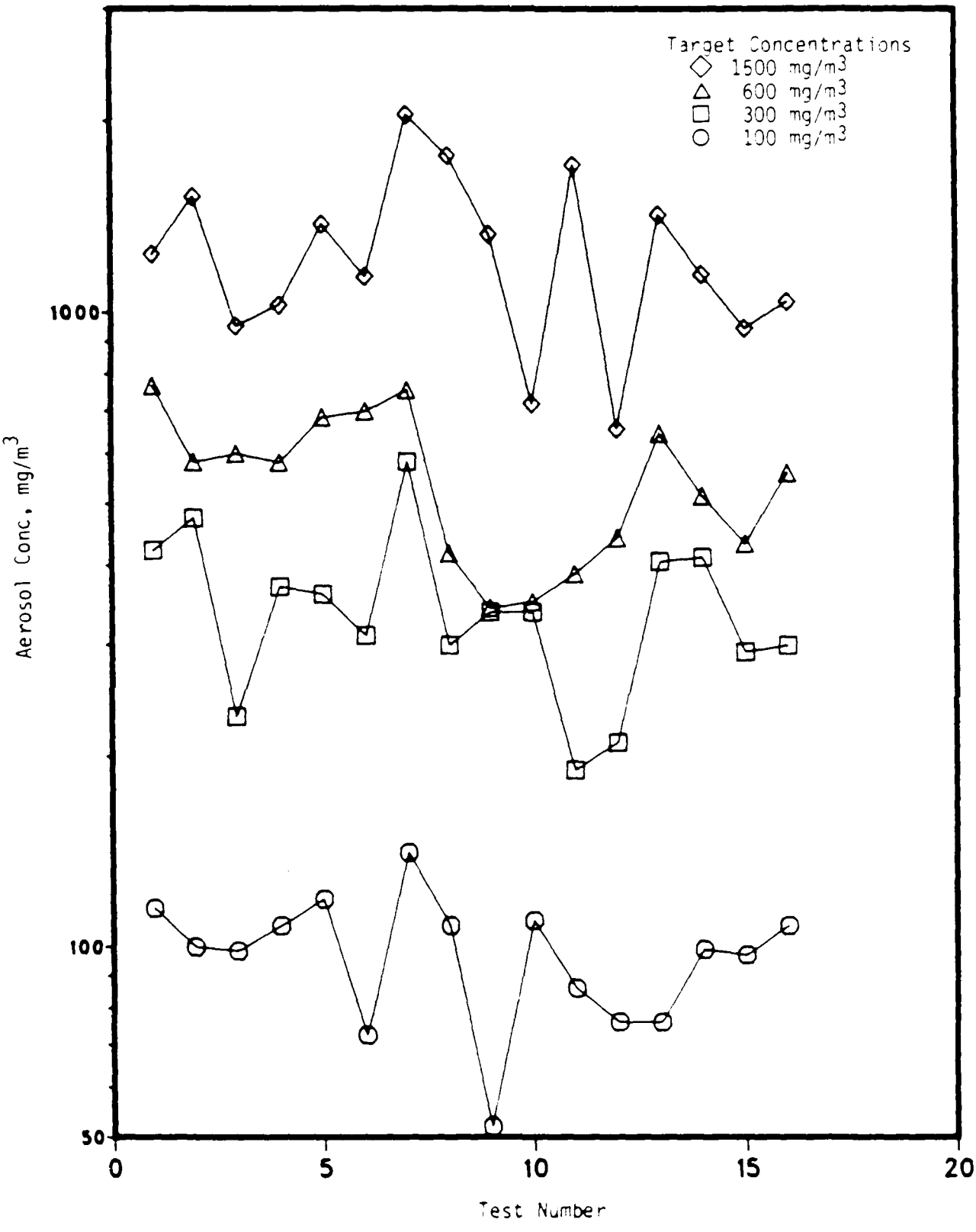
In addition to the measurements performed for each of the exposures to characterize the principal exposure parameters, ancillary measurements were performed in order to verify that the microcosms were not exposed to significant concentrations of combustion products. To this end, samples were withdrawn from one of the chambers at the 1500 mg/m^3 target aerosol concentration and analyzed for CO, NO, and NO_2. The CO was determined to be present at approximately 6 ppm using a Beckman 6800 total carbon analyzer. The NO_2 was found to be less than 10 ppb and NO at about 20 ppb using a chemiluminescent detector. These values are all well below values which are physiologically significant for the microcosms, especially considering the short duration of the exposures[40,41,42].

Limited measurements of the aerosol size distribution produced by the phosphorus smoke materials were made at the high concentration level also to insure that the size of the particles was consistent with measurements reported by others. Samples were collected on millipore filter substrates using a cascade impactor and analyzed gravimetrically. The distribution was seen to have an aerodynamic mass median diameter (AMMD) of 1.0 to 1.2 μm and a geometric standard deviation (σg) of 1.5 to 1.7. These values are similar to measurements of AMMD = 0.7 to 1.0 μm and σg = 1.45 reported by Steubing, et al.[43] for slightly lower concentrations. Ballou et al.[1] reported AMMD values of 0.9 to 1.4 μm and σg = 1.5 to 1.7 for smokes of RP/BR at similar and higher concentrations, and similar values were also reported by Burton et al.[4]. Samples were also collected at the lowest target concentration levels used in these exposures, but there proved to be too little material present for reliable analysis.

3.3.1.1 Aerosol Concentration Measurements. As indicated in Section 2.4, the aerosol mass concentration in each of the exposure chambers was measured just after the end of the aerosol generation period. This measurement was used as a check on the consistency of the aerosol generated from the measured initial masses of smoke material. The actual masses of material burned to achieve the target exposure levels are given in Table 5. This material was ignited and permitted to burn until the flame was extinguished. The material was dispersed as evenly as possible in the combustion pans for all exposures, but there were still some variations in the combustion efficiency. These aerosol mass concentration measurements are presented in graphical form in Figures 5 through 8 for each of the exposures performed. Note that the lines used to interconnect the data points are present only to clarify which values are associated with which microcosm group and do not convey any other information. In an ideal situation, these figures would each contain four horizontal lines. There clearly is variability in these measured values of the aerosol mass concentration. Repeated measurements of a given aerosol sample would yield values which vary by only ±10 percent, approximately. The sampling of a small aliquot of the chamber volume introduces the possibility of variation in the data, for while this procedure disturbs the sampled atmosphere to the least extent possible, it permits measurement of only a small volume which may not be representative of the average value for the entire chamber atmosphere. The mixing fans were installed in the chamber,

TABLE 5. AMOUNT OF MATERIAL BURNED TO ACHIEVE TARGET CONCENTRATION

Target (mg/m^3)	WP (g)	RP/BR (g)
100	0.2	0.2
300	0.5	0.5
600	1.1	1.1
1500	2.6	2.4


Target Concentrations
1500 mg/m3
600 mg/m3
300 mg/m3
100 mg/m3
1000
100
50
Aerosol Conc, mg/m3
0
5
10
15
20
Test Number

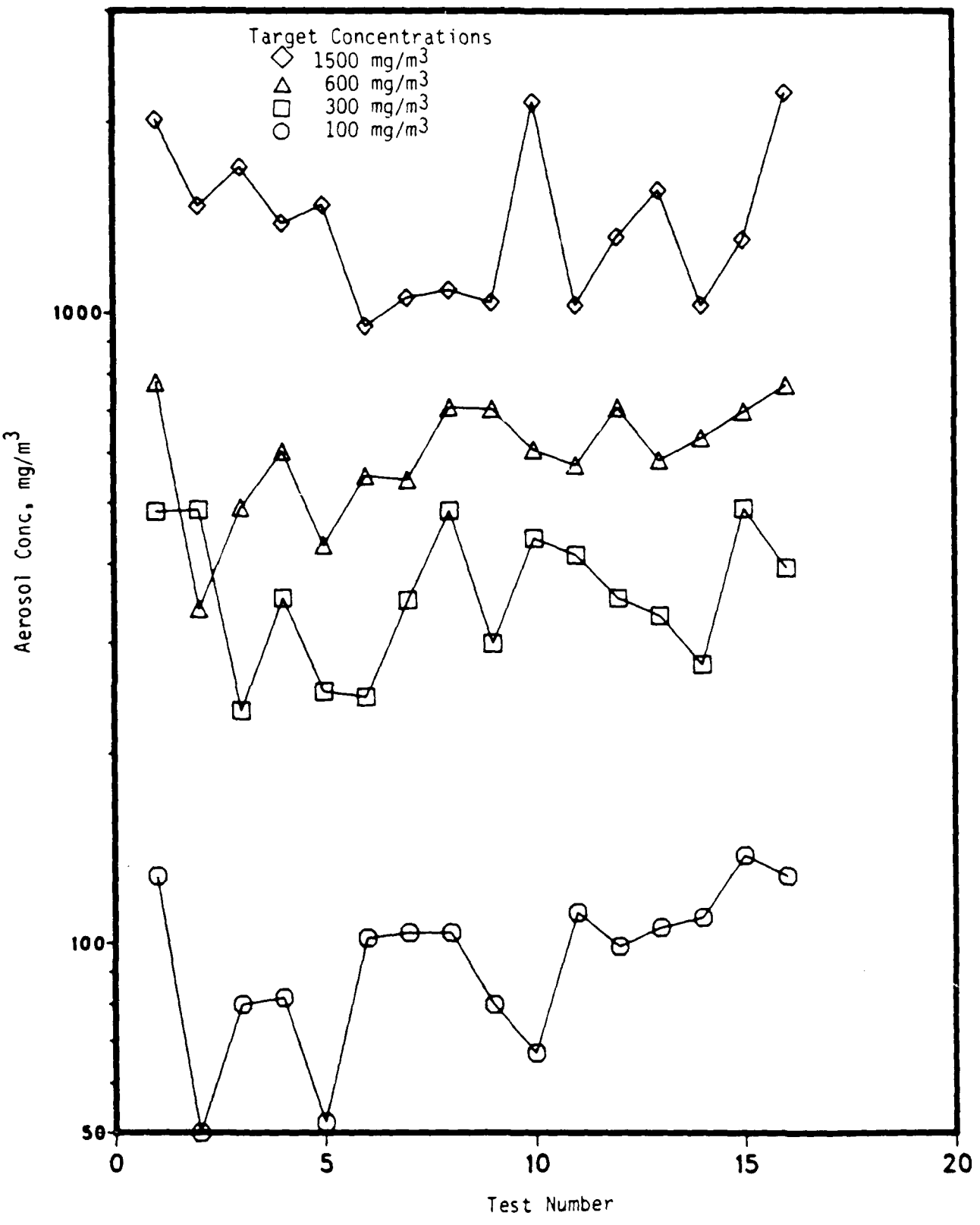


FIGURE 6. INITIAL AEROSOL MASS CONCENTRATION FOR RP BR EXPOSURES IN DOSE GROUP B

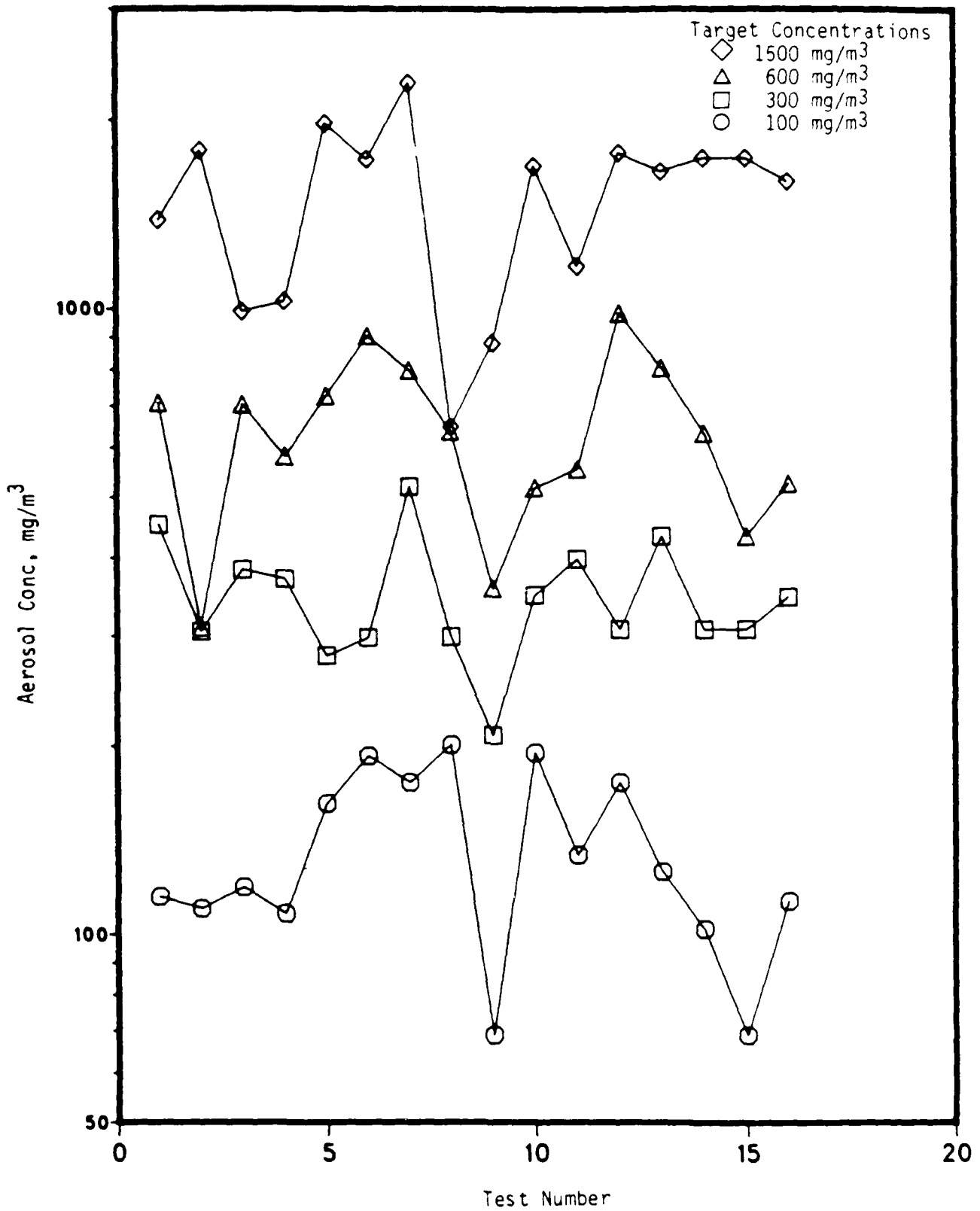


FIGURE 7. INITIAL AEROSOL MASS CONCENTRATION FOR nP EXPOSURES IN DOSE GROUP A

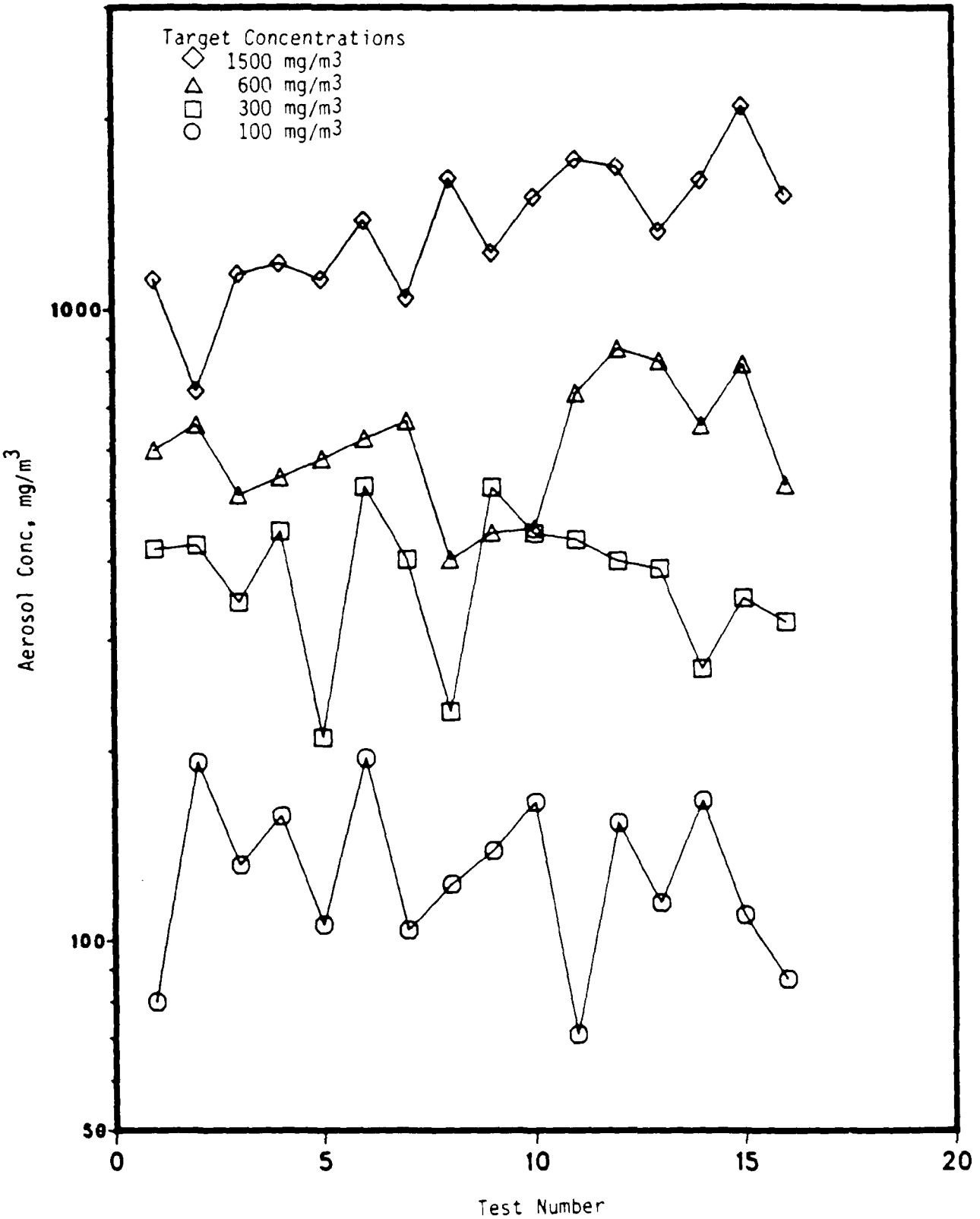


FIGURE 8. INITIAL AEROSOL MASS CONCENTRATION FOR AP EXPOSURES IN DOSE GROUP B

and a delay between aerosol generation and sampling was included in the protocol to help provide for representative sampling, but some variability in the measured aerosol mass concentrations clearly remained. An alternate potential cause for the variability observed is variation from test to test in the actual mass of smoke obtained, either as a result of different efficiencies of combustion or of different particle sizes obtained due to different relative humidity values. This possibility is discussed further in connection with the deposition coupon results.

3.3.1.2 Phosphorus Analysis of Deposited Aerosol. Results from the colorimetric and titrimetric procedures for determining the H_3PO_4-P concentration in the deposited smoke aerosol are compared in Table 6 and Figure 9. The correlation coefficient (Figure 9) for comparing the methods was determined to be 0.996. The close correlation between the two methods allows satisfactory quantitation of H_3PO_4-P loadings on the deposition coupons.

Results from the comparative analysis of the colorimetric method versus the titration method validate the use of the titration procedure for quantifying mass loadings of phosphorus aerosols on deposition coupons. The pH end point of 9.0 was determined to be satisfactory for phosphorus in the deposition coupons by comparing samples analyzed by titration to identical samples analyzed by the colorimetric method.

TABLE 6. COMPARISON OF THE COLORIMETRIC METHOD TO THE TITRATION METHOD FOR DETERMINATION OF H_3PO_4-P

Sample	Colorimetric Method, mg P	Titration Method, mg P
1	0.96	0.69
2	21.7	23.6
3	0.71	0.50
4	0.46	0.19
5	0.52	0.12
6	2.14	1.69
7	3.10	3.69
8	16.2	15.4

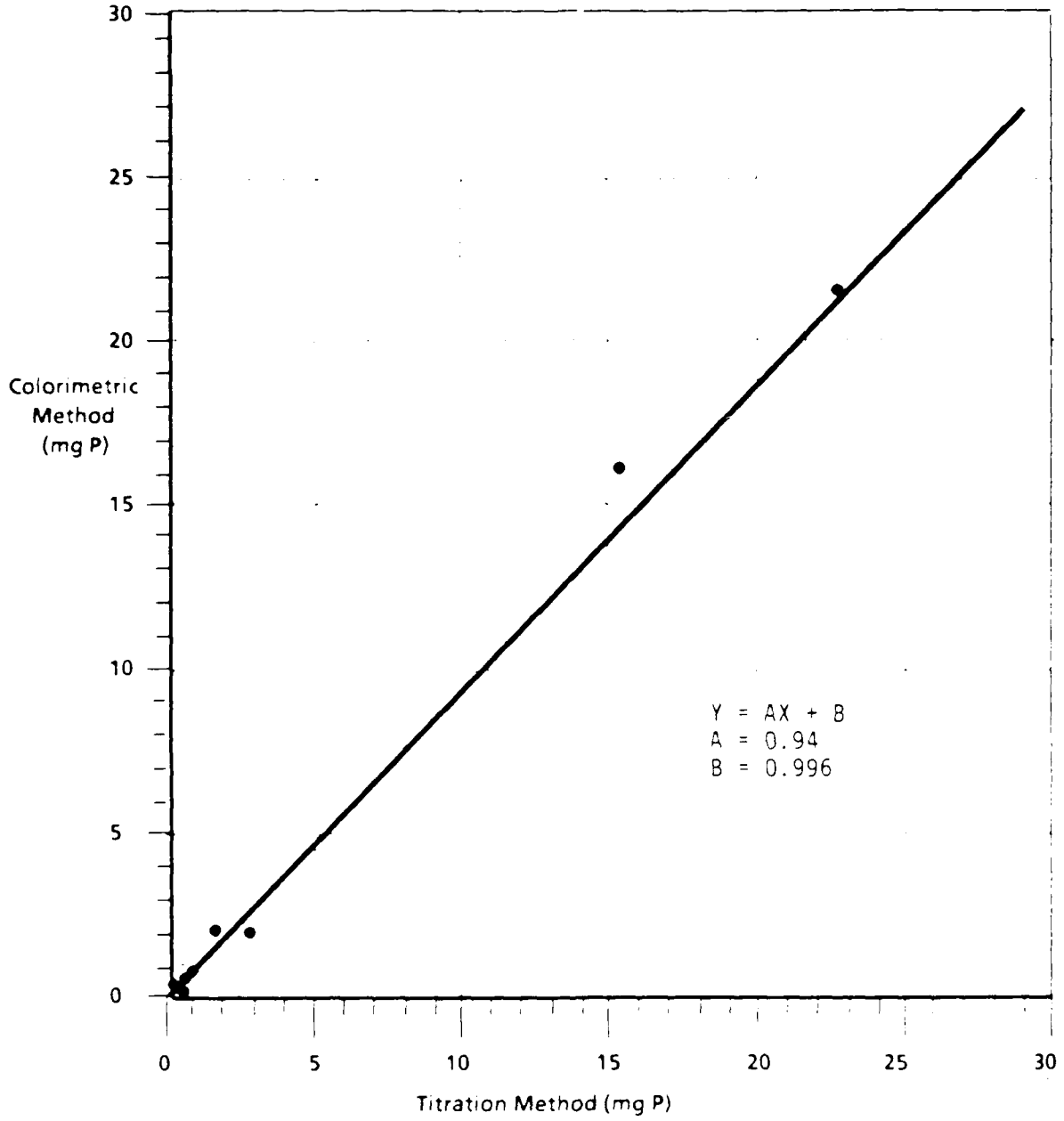


FIGURE 9. COMPARISON OF THE COLORIMETRIC METHOD TO THE TITRATION METHOD FOR DETERMINATION OF H_3PO_4-P

3.3.1.3 Deposited Aerosol Measurements. The dosage which is actually applied to the microcosms is most closely related to the aerosol mass deposited on horizontal surfaces in the exposure chambers. Since this is an integral measurement of the deposition on the coupon surfaces over the course of the entire exposure, it is not strongly affected by inhomogeneities which exist in the chamber atmosphere over short periods of time. It should be pointed out that different microcosms, and different plants within each microcosm, will be subject to different dosages from the same exposure due to differences in their amount of horizontal leaf area (see Section 3.3.5.1).

The measurements of deposited phosphorus mass, as described above, are presented in Tables 7 and 8. As is clear in these tables, there is variability in the applied dosages as measured by this technique. This variability is attributable to variance in the chemical determination performed and in the efficiency of the phosphorus combustion. The means and standard deviations for each exposure level are provided in Tables 7 and 8 as summary statistics to describe the relative dosage levels.

TABLE 7. TITRATION ANALYSIS FOR PHOSPHORUS ON DEPOSITION COUPONS (PETRI PLATES) FROM RP/BR SMOKE EXPOSURES

Test Number	Quantity ($\mu g/cm^2$) of Phosphorus on Deposition Coupons							
	Group A Target Concentrations (mg/m^3)				Group B Target Concentrations (mg/m^3)			
	100	300	600	1500	100	300	600	1500
1	6.9	19	53	165	6.9	18	44	198
2	3.5	18	51	169	4.2	20	43	167
3	3.5	15	43	178	3.5	15	40	171
4	2.8	19	45	182	4.2	21	45	164
5	2.8	23	52	199	2.8	15	37	222
6	2.8	17	42	193	3.5	13	73	187
7	4.2	14	38	191	2.8	15	38	200
8	4	13	43	128	3.5	18	43	128
9	4.9	21	57	190	4.9	21	64	158
10	4.9	27	64	131	4.9	24	46	159
11	4.9	27	64	131	4.9	24	46	159
12	2.8	14	34	168	3.5	13	38	170
13	2.8	15	44	173	3.5	17	41	157
14	4.2	16	35	148	2.8	13	36	157
15	3.5	14	39	187	3.5	12	31	144
16	3.5	12	40	168	2.8	13	33	150
Mean	3.9	18	46	169	3.9	17	44	168
Standard Deviation	±1.1	±4.7	±9.4	±23.1	±1.1	±4.0	±11	±23

TABLE 8. TITRATION ANALYSIS FOR PHOSPHORUS ON DEPOSITION COUPONS (PETRI PLATES) FROM WP SMOKE EXPOSURES

Test Number	Quantity ($\mu g/cm^2$) of Phosphorus on Deposition Coupons							
	Group A Target Concentrations (mg/m^3)				Group B Target Concentrations (mg/m^3)			
	100	300	600	1500	100	300	600	1500
1	5.6	23	54	128	6.9	22	41	143
2	7.7	24	24	136	6.3	24	65	136
3	6.9	26	52	148	4.9	25	50	143
4	5.6	22	52	133	6.9	24	58	154
5	5.6	27	59	148	6.3	23	57	141
6	6.3	23	51	147	6.9	24	24	107
7	6.3	23	34	99	3.5	11	24	73
8	5.6	9.8	22	82	4.2	9.1	23	92
9	3.5	8.4	23	82	6.3	17	37	120
10	6.3	17	34	122	6.9	13	26	125
11	8.4	17	45	133	3.5	13	36	143
12	7.7	17	49	150	5.6	14	36	136
13	3.5	14	36	131	3.5	9.1	34	134
14	4.2	10	34	136	4.9	8.3	32	134
15	4.2	7.7	31	133	3.5	8.3	29	127
16	3.5	9.1	29	133	4.2	8.3	311	122
Mean	5.7	17.4	39	128	5.3	16	38	127
Standard Deviation	±1.6	±6.8	±12	±22	±1.4	±6.7	±13	±21

3.3.2 Nutrient Loss in Soil Leachate

Soil leachates from microcosms exposed to RP/BR or WP were analyzed for nutrient concentrations as discussed in Section 2.4.8.1. These analyses were meant to serve as indicators of plant stress and toxicological effects due to smokes exposure. If the pool of nutrients in the soil is depleted by leaching and not replaced (e.g., by agricultural fertilization practices), plants eventually will be unable to obtain enough nutrients for normal growth. Leachates were collected for analyses on three dates. The first and second leachates were collected during the 8-week exposure period, while the third leachate was collected three weeks after the last exposure. The first leachates were analyzed for total organic carbon (TOC), nitrate-nitrogen (NO_3-N), ammonium-nitrogen (NH_4-N), calcium (Ca), and potassium (K). Subsequent leachates were monitored for NO_3-N and Ca. The means and standard deviations for the weight of each selected nutrient lost in each of three leachate collection periods and at each dose level of both smokes are presented in Appendix B, Tables B-1 to B-3.

Analysis of the first set of leachates showed that NH_4-N concentrations were similar to those in distilled water. This occurred despite the smoke type or exposure dose. Consequently, subsequent leachates were not analyzed for NH_4-N. Ammonium-nitrogen was initially selected for monitoring in the first leachate because of its importance as a plant nutrient and its relative immobility in soil.

Total organic carbon (TOC) and K were analyzed in the first leachates from the microcosms. The actual quantity of TOC and K lost in the leachates was determined by multiplying the leachate volume by the concentration of the nutrient in the leachate. Neither a two-way ANOVA (cart x dose) nor the linear term from the regression equation on dose indicated any statistically significant effect due to exposure to either smoke.

As mentioned previously, Ca and NO_3-N were monitored in all three leachate collections. After each collection, the volume of leachate was multiplied by the concentration of the nutrient in the leachate in order to determine the actual quantity of nutrient lost. Cumulative losses were determined by summing the losses in individual leachates. These data were analyzed individually and cumulatively. Analysis of the sum of the three

leachates included an ANOVA with target dose as the factor, and a quadratic regression with actual deposition of phosphorus as the dependent variable. When the quadratic term of the regression was not significant, a linear regression was fitted. The analyses showed no statistically significant response to either smoke for total NO_3-N. However, the quadratic term of the regression equation did show a statistically significant effect ($p < 0.01$) of RP/BR smoke (Figure 10), but no significant effect of WP, for total Ca. These data are summarized in Table 9.

These data indicate that exposure of the soil-plant system to relatively high concentrations of RP/BR or WP smokes over an 8-week period had little if any detrimental impact. This conclusion is based on earlier observations that terrestrial ecosystems subjected to some physical or chemical stress may lose their capacity to conserve critical nutrients. Likens et al. (1970) showed that large forested ecosystems that were clear-cut and treated with herbicides to prevent regrowth lost substantial amounts of nutrients to groundwater, especially NO_3-N and Ca. Other researchers over the past decade have shown that losses of selected nutrients in soil water or leachate may be good indicators of system disruption[31,44,45]. Cumulative nutrient losses in leachates from soil-core microcosms that were very similar to those employed in the current research project proved to correlate well with decreases in plant productivity when soil was amended with toxic levels of fly ash[12]. In those instances where fly ash was added in nontoxic amounts, nutrient losses and plant productivity were similar to untreated controls.

Based on nutrient loss measurements, the current data predict that the obscurant smokes should have little short-term (3 months) impact on the soil-plant system. These predictions were substantiated by biomass yield measurements (see Section 3.3.3) and by soil (microbial) respiration experiments (see Section 3.4). However, as mentioned above, the quadratic term of the regression equation indicated a significant loss of Ca at the highest exposures to RP/BR. As will be seen in the following section, the yield of sweetclover was significantly depressed at the corresponding exposure to RP/BR. Thus, the increased Ca loss may have resulted from depressed Ca uptake by the sweetclover. In general, legumes require considerably more Ca than nonlegumes[46], so a reduction in sweetclover growth could manifest as increased Ca loss.

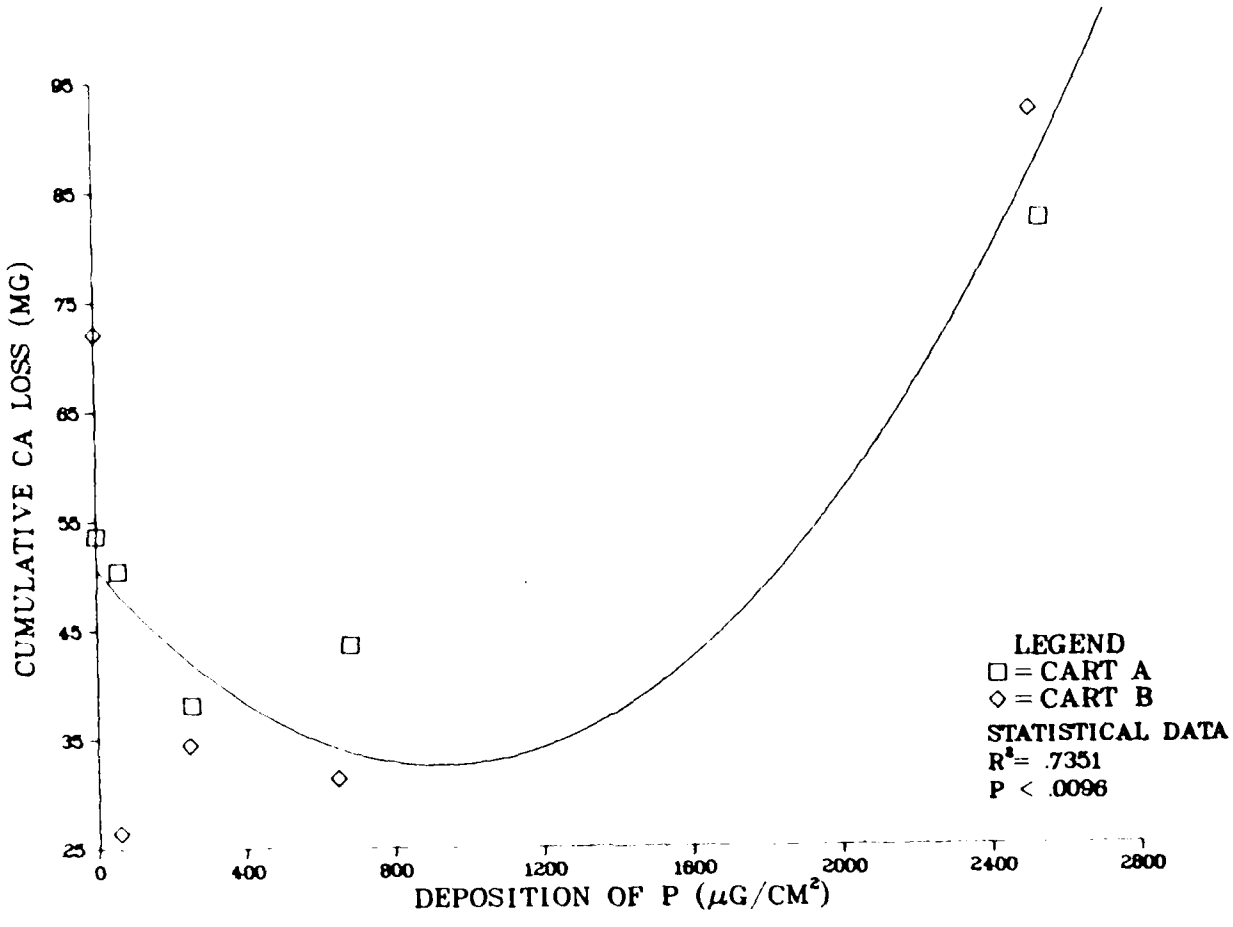


FIGURE 10. QUADRATIC REGRESSION OF CUMULATIVE LOSS OF CALCIUM (mg) FROM MICROCOSMS EXPOSED TO RP/BR SMOKE VERSUS TOTAL PHOSPHORUS DEPOSITED IN THE RANGE-FINDING TEST

TABLE 9. STATISTICAL ANALYSIS OF NUTRIENT (NO_3 AND CA) LOSS DATA IN LEACHATE FROM MICROCOSMS EXPOSED TO TWO PHOSPHORUS SMOKES

Nutrient Concentration in Leachates	RP/BR Smoke			WP Smoke		
	ANOVA (on Target Dose)	Linear Regression[a] (on P Deposition)	Quadratic Regression (on P Deposition^2)	ANOVA (on Target Dose)	Linear Regression[a] (on P Deposition)	Quadratic Regression (on P Deposition^2)
Nitrate (1st + 2nd Leachate)	NSIG[b]	NSIG	NSIG	NSIG	NSIG	NSIG
Nitrate (1st + 2nd + 3rd Leachate)	NSIG	NSIG	NSIG	NSIG	NSIG	NSIG
Calcium (1st + 2nd Leachate)	NSIG	NSIG	NSIG	NSIG	NSIG	NSIG
Calcium (1st + 2nd + 3rd Leachate)	NSIG ($p = .062$)	No Test Required	SIG[c] at .01 Level	NSIG	NSIG	NSIG

(a) Linear regression curve was fitted when the quadratic term of the quadratic regression was not significant; otherwise linear regression analysis was not required.

(b) NSIG = nonsignificant ($p > 0.05$).

(c) SIG = significant.

3.3.3 Biomass Yield

Above-ground plant parts were harvested, oven-dried, and weighed (see Section 2.4.8.2) as an indicator of ecosystem health with and without exposure to RP/BR or WP smoke. The smoke aerosols have the potential for affecting biomass yield both (1) directly through leaf exposure and (2) indirectly through soil deposition effects on soil organisms, nutrient cycling, and availability of toxic elements.

Biomass yield data from each of the two harvests were analyzed separately and as a combined total for both harvests. Data from the first harvest were only analyzed as the total for all species combined. In the second harvest, however, the biomass for each species was analyzed separately and as a total for all species. The mean and standard deviation of biomass yield data for each dose level of each biomass parameter and both harvests are presented for both smokes in Appendix B, Tables B-4 to B-6.

Biomass data from the first harvest (Appendix B, Table B-4) did not show any significant effects due to exposure to either RP/BR or WP smoke. The effects of smoke exposure on biomass from the first harvest were analyzed by a two-way ANOVA (cart x dose) and the linear term from a regression equation on dose. In no case was there a statistically significant effect of dose for either RP/BR or WP smoke ($p < 0.05$).

Biomass data from the second harvest were statistically analyzed for effects of smoke dose on individual species (wheat, ryegrass, and sweetclover) and on the combined biomass for both harvests for all species (Appendix B, Tables B-5 to B-6). Analysis included ANOVA and Bonferroni's tests using target dose as the factor, and quadratic or linear regression with deposition of phosphorous as the dependent variable (Table 10). Evaluation of the linear and quadratic terms of regression equations indicated that neither smoke produced a significant effect on the combined biomass (both harvests) for all species or on ryegrass biomass (second harvest).

Both smokes produced a signficant effect on sweetclover biomass (Table 10), as shown by the quadratic regressions fitted to the biomass data in Figures 11 and 12. In addition, ANOVA indicated a signficant effect of dose on sweetclover biomass for both smokes. Bonferroni's test indicated that the 300 and 1500 mg/m^3 dose groups of WP were significantly different from the

TABLE 10. STATISTICAL ANALYSIS OF BIOMASS YIELD FROM MICROCOSMS EXPOSED TO TWO PHOSPHORUS SMOKES

Biomass (Mean Dry Weight)[a]	RP/BR Smoke			WP Smoke		
	ANOVA (on Target Dose)	Linear Regression[b] (on P Deposition)	Quadratic Regression (on P Deposition^2)	ANOVA (on Target Dose)	Linear Regression[b] (on P Deposition)	Quadratic Regression (on P Deposition^2)
Wheat Biomass Second Harvest	NSIG[c]	NSIG	NSIG	SIG[d] at .05 Level (None)[e]	SIG at .05 Level[g]	SIG at .05 Level (quadratic term not significant p = 0.056)[g]
Ryegrass Biomass Second Harvest	NSIG	NSIG (p = .089)	NSIG	NSIG	NSIG	NSIG
Sweetclover Biomass Second Harvest	NSIG	No Test Required	SIG at .05 Level	SIG at .01 Level (Dose 3, 15)[f]	No Test Required	SIG at .001 Level
Total Biomass Both Harvests	NSIG	NSIG	NSIG	NSIG (p = .097)	NSIG	NSIG

(a) Plant dry weight data are from the second harvest except for total biomass, which sums both harvests for all species.
(b) Linear regression curve was fitted when the quadratic term of the quadratic regression was not significant; otherwise, linear regression analysis was not required.
(c) NSIG = nonsignificant.
(d) SIG = significant.
(e) No dose groups significantly different from control by Bonferroni's test.
(f) These two dose groups were significantly ($p < 0.05$) different from 0 by Bonferroni's test.
(g) Based on the r^2 values the quadratic regression fits better.

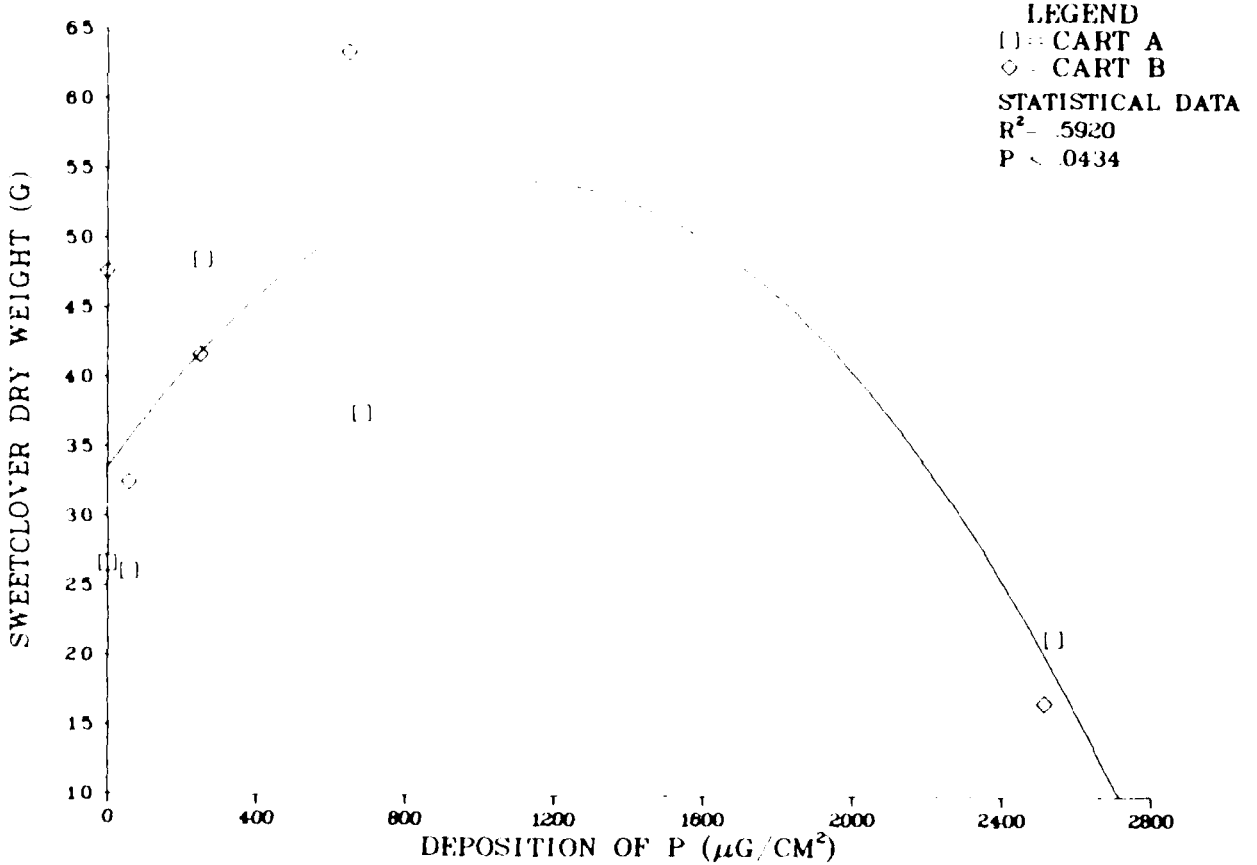


FIGURE 11. QUADRATIC REGRESSION OF SWEETCLOVER BIOMASS FROM THE SECOND HARVEST OF MICROCOSMS EXPOSED TO RP/BR SMOKE VERSUS TOTAL PHOSPHORUS DEPOSITED IN THE RANGE-FINDING TEST

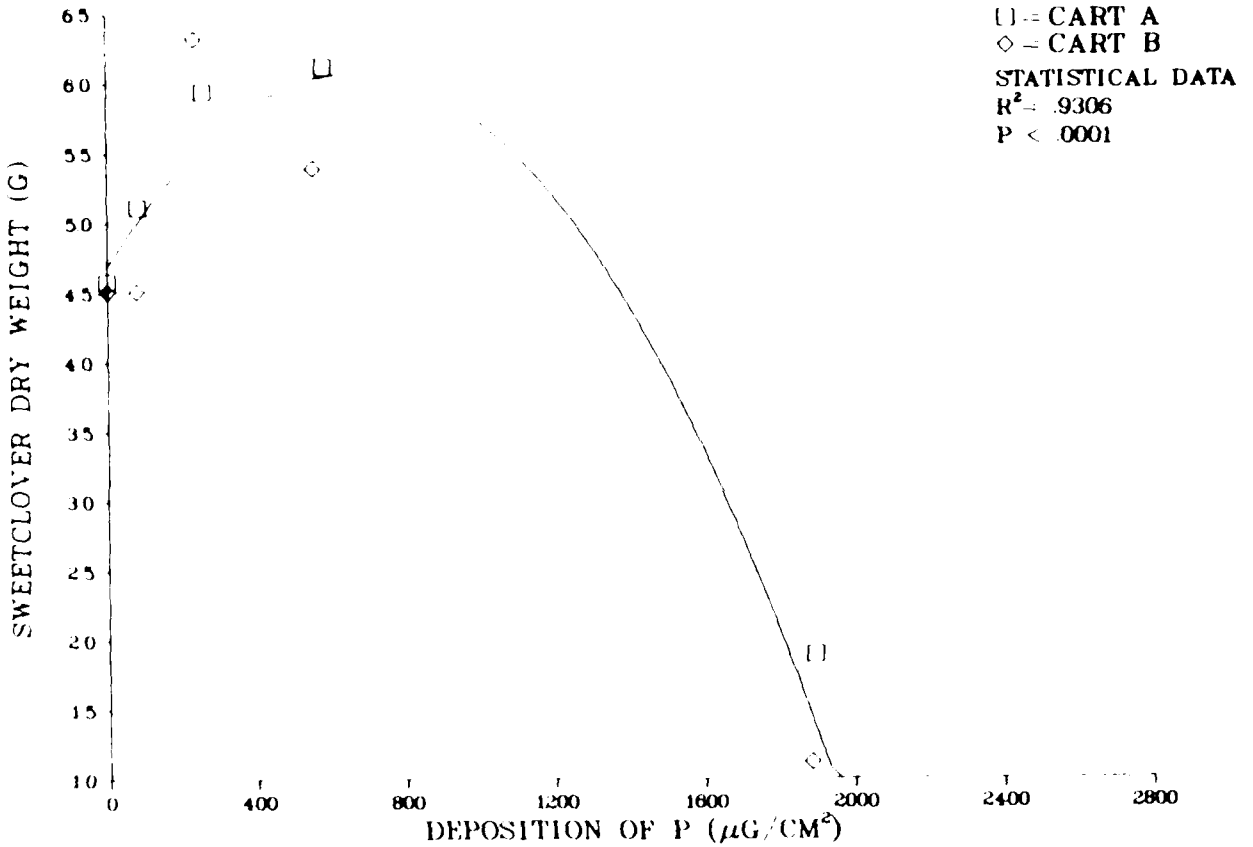


FIGURE 12. QUADRATIC REGRESSION OF SWEETCLOVER BIOMASS FROM THE SECOND HARVEST OF MICROCOSMS EXPOSED TO WP SMOKE VERSUS TOTAL PHOSPHORUS DEPOSITED IN THE RANGE-FINDING TEST

control for sweetclover biomass. As indicated in Figures 11 and 12, the middle dose group showed improved yield relative to controls, while the high dose group showed a reduced yield relative to controls.

For wheat biomass, the two statistical methods showed significant effects of dose due to WP smoke exposure, but not for RP/BR smoke exposure (Table 10). Although the regression equation fitted to wheat biomass data from WP exposure (Figure 13) shows an increase in yield relative to controls, this trend was not confirmed by Bonferroni's test. According to Bonferroni's test, no dose groups for wheat biomass were significantly different from the controls. Based on the superior R^2 value, a quadratic regression was selected to fit the wheat biomass data from the WP smoke exposure rather than a linear regression, even though the quadratic term was not significant at $p = 0.056$.

The possible improvement in wheat biomass exposed to the highest WP smoke exposure compared to controls (see Figure 13) may be due to increased As supplied as a contaminant in the WP. Calcium arsenate has been shown to increase wheat biomass[47]. A similar trend for improved wheat biomass at the highest RP/BR exposure level was not detected by regression analysis, in spite of the fact that more total P was deposited (see Section 3.3.1.3) and more As was taken up by plants (see Section 3.3.4) at the highest RP/BR dose than at the highest WP dose. It is possible that the As levels in plant tissue (2.55 ppm) at the highest RP/BR smoke treatment level were beyond the stimulatory stage for wheat and beginning to reach toxic levels, while As concentrations in plant tissue (0.9 ppm) at the highest WP smoke treatment level were in the stimulatory range for wheat. Arsenic tissue levels of 0.15 to 0.30 ppm in wheat grain are considered intermediate between deficiency and toxicity[47].

The sweetclover biomass may have been affected more than the other species for two reasons. First, the sweetclover appeared to have the greatest horizontal leaf surface area; thus, it would be expected to receive more smoke aerosol deposition than the other two plant species. This possibility is supported by the preliminary stress-ethylene test, where sweetclover had the greatest increasing slope for the dose-response curve, compared to the other species (see Section 3.1.2). Wheat had almost equally as large of total surface area (see Section 2.4.9.1), but the leaves were oriented in the vertical position. Second, legumes like sweetclover are extremely sensitive to As[47], which others have analyzed in the unburned WP at a concentration of 84 ppm [3]. This study did not include the elemental analysis of unburned smoke material,

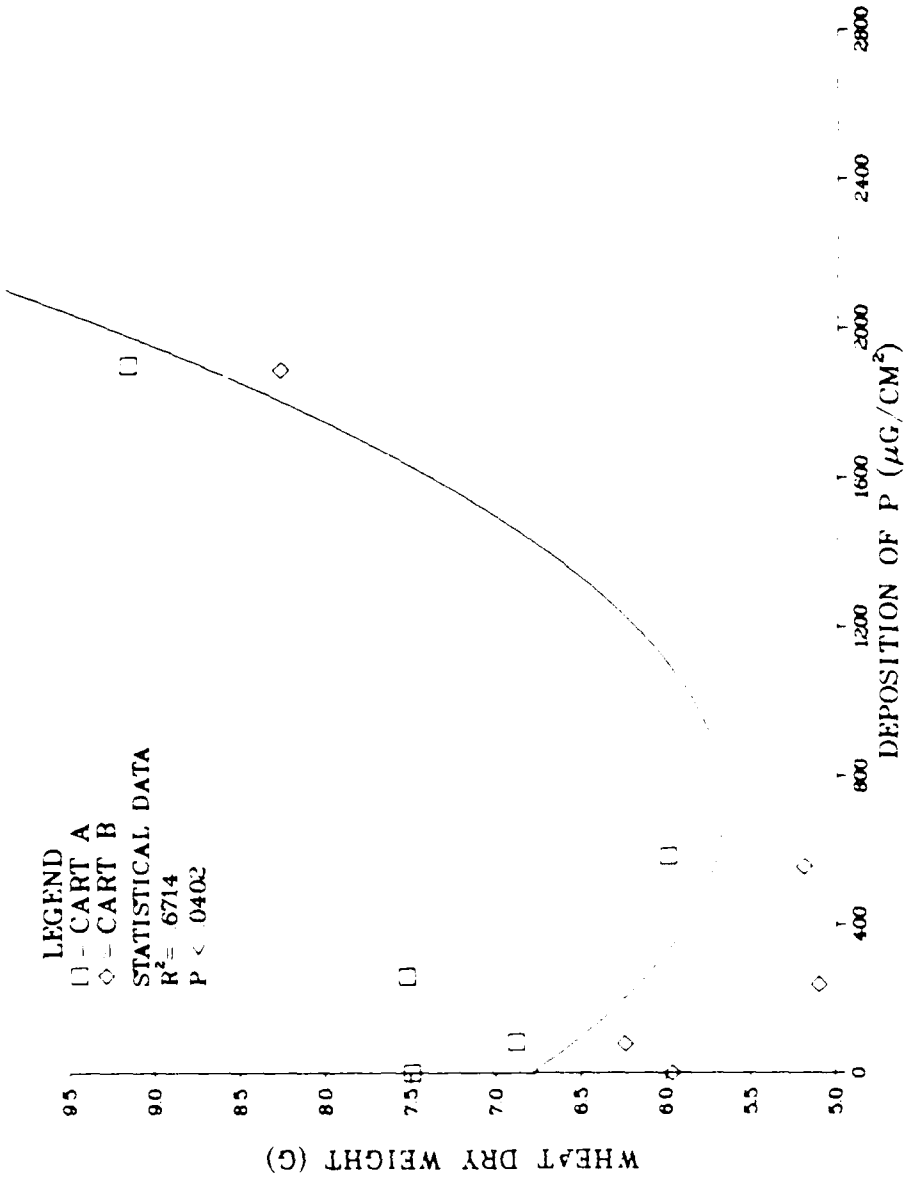


FIGURE 13. QUADRATIC REGRESSION OF WHEAT BIOMASS FROM THE SECOND HARVEST OF MICROCOSMS EXPOSED TO WP SMOKE VERSUS TOTAL PHOSPHORUS DEPOSITED IN THE RANGE-FINDING TEST

because the chemistry of the two phosphorus smokes has been extensively evaluated through other Army contracts[1,2,3]. The As level in unburned WP determined by Katz et al.[3] is an order of magnitude above typical background levels in the soil[50]. Arsenic toxicity will be discussed in more detail under the following section.

3.3.4 Element Uptake by Plants

Above-ground plant parts were ground, digested, and chemically analyzed (see Section 2.4.8.3) for concentrations of selected nutrients, to determine if exposure to RP/BR or WP smoke changed the element concentrations in exposed plants relative to controls. These element concentration data were used to evaluate the potential for toxicity or deficiency problems in plants and the potential for toxicity to consuming animals, including grazing livestock and man.

Data on plant uptake of 24 elements from the first harvest were analyzed statistically, in order to make a decision on which 6 elements would be chemically analyzed in the second harvest. In both cases, the statistics involved analyses by a two-way ANOVA (cart x target dose) and linear regression on either target dose (first harvest) or total P deposition (second harvest). The mean and standard deviation of element uptake concentrations in each type of plant tissue for each dose level and both harvests are presented for both smokes in Appendix B, Tables B-7 to B-9.

Statistical analysis of data on plant tissue concentrations showed effects of one or both smokes on P, As, Pb, and Zr (Table 11). Both statistical methods for the first harvest showed very strong effects ($p < 0.001$) of both RP/BR and WP smokes on uptake of the elements P and As. The ANOVA also showed a significant effect ($p < 0.01$) of the WP smoke on uptake of Al and Pb. The linear term of a regression equation showed a significant effect ($p < 0.05$) of the RP/BR smoke on uptake of Zr. Neither of the two statistical methods indicated any statistically significant effect ($p > 0.05$) of exposure to either smoke of any other metal.

The choice of elements selected for analysis in the second harvest were based on: (1) statistical analysis of uptake data for 24 elements analyzed in plant tissue from the first harvest, and (2) data in the literature on the relative toxicity of these 24 elements to terrestrial plants and animals[48,49,50]. As discussed above, statistical analysis showed

significant ($p < 0.05$) effects of one or both phosphorus smokes on plant uptake of P, As, Al, Pb, and Zr. Uptake data for three elements (Cr, Mo, and V) analyzed by both ANOVA and evaluation of the linear term of a regression equation had p values between 0.05 and 0.10, which were due to more than a single increased concentration (Table 11). Of these latter three elements, Cr and Mo were chosen because of their relatively high toxicity to plants or animals. Zirconium was not analyzed even though there was significant uptake in plants, because of the element's relatively low toxicity. Although P is not particularly toxic, statistically significant effects of both smokes on plant uptake of P were detected.

Statistical analysis of plant concentration data for six elements determined from the second harvest showed significant effects of one or both smokes on uptake of As, P, Al, and Pb (Table 12). Plant uptake of Al and Pb was significantly affected by WP smoke, based on the linear term of a regression equation, but this trend was not detected by ANOVA. Neither of the two statistical methods indicated any significant effect ($p > 0.05$) of exposure to either smoke on Cr or Mo uptake or of exposure to RP/BR smoke on Al or Pb uptake.

The relationship between element uptake of the six elements and exposure was adequately described by a straight line (Table 12), except for As uptake and WP smoke exposure, where a quadratic regression was the best fit. Statistically significant regressions of element uptake on smoke exposure are shown in the following figures: As uptake with RP/BR smoke (Figure 14), As uptake with WP smoke (Figure 15), P uptake with RP/BR smoke (Figure 16), P uptake with WP smoke (Figure 17), Al uptake with WP smoke (Figure 18), and Pb uptake with WP smoke (Figure 19).

Uptake of Al, As, and Pb by plants exposed to the phosphorus smokes is probably a result of their presence as impurities in the unburned phosphorus material used in the smokes. Katz, et al.[3], reported the following elemental impurities in WP: Al-20 ppm, As-84 ppm, and Pb-1.27 ppm. Ballou[1] reported that RP also contains trace impurities. The levels of trace impurities in the batches of RP/BR and WP used in this project were not determined.

The As levels in plant tissue exposed to the smokes (up to 2.55 ppm; see Appendix B, Table B-9) were above the normal (1 ppm) level reported by Allaway[50], and may have affected biomass of sweetclover and wheat. Significant declines in sweetclover biomass at high treatment levels for both

TABLE 11. STATISTICAL EVALUATION OF CONCENTRATION DATA FOR 25 ELEMENTS IN PLANT TISSUE FROM THE HARVEST OF MICROCOSMS EXPOSED TO TWO PHOSPHORUS SMOKES

Element	RP/BR Smoke		WP Smoke	
	ANOVA (by Cart X Target Dose)	Linear Regression (by Target Dose)	ANOVA (by Cart X Target Dose)	Linear Regression (by Target Dose)
Al	NSIG[a]	NSIG	SIG[b] 0.1 Level	NSIG
B	NSIG	NSIG	NSIG	NSIG
Ba	NSIG	NSIG	NSIG	NSIG
Ca	NSIG	NSIG	NSIG	NSIG
Cd	All Values Identical	All Values Identical	All Values Identical	All VAlues Identical
Co	All Values Identical	All Values Identical	NSIG	NSIG
Cr	NSIG	NSIG (p = .095)	NSIG	NSIG
Cu	NSIG	NSIG	NSIG	NSIG
Fe	NSIG	NSIG	NSIG	NSIG
Mg	NSIG	NSIG	NSIG	NSIG
Mn	NSIG	NSIG	NSIG	NSIG
Mo	NSIG	NSIG	NSIG (p = .097)	NSIG
Na	NSIG	NSIG	NSIG	NSIG

TABLE 11. STATISTICAL EVALUATION OF CONCENTRATION DATA FOR 25 ELEMENTS IN PLANT TISSUE FROM THE HARVEST OF MICROCOSMS EXPOSED TO TWO PHOSPHORUS SMOKES (Continued)

Element	RP/BR Smoke		WP Smoke	
	ANOVA (by Cart X Target Dose)	Linear Regression (by Target Dose)	ANOVA (by Cart X Target Dose)	Linear Regression (by Target Dose)
Ni	NSIG[a]	NSIG	NSIG	NSIG
P	SIG[b] at .0001 Level	SIG at .0001 Level	SIG at .0001 Level	SIG at .0001 Level
Pb	NSIG	NSIG	SIG at .01 Level	NSIG
Sr	NSIG	NSIG	NSIG	NSIG
Ti	NSIG	NSIG (p = .058)[a]	All Values *Identical*	All Values *Identical*
Tl	NSIG	NSIG	NSIG	NSIG
V	NSIG	NSIG	NSIG (p = .091)	NSIG
Yt	NSIG	NSIG (p = .071)[c]	NSIG	NSIG
Zr	NSIG	SIG at .05 Level	NSIG	NSIG
As	SIG at .0001 Level	SIG at .0001 Level	SIG at .0001 Level	SIG at .0001 Level
Se	NSIG	NSIG (p = .058)[c]	All Values Identical	All Values Identical

(a) NSIG = nonsignificant.
(b) SIG - significant.
(c) Low p value likely due to a single high value.

TABLE 12. STATISTICAL EVALUATION OF CONCENTRATION DATA FOR SIX ELEMENTS IN PLANT TISSUE FROM THE SECOND HARVEST OF MICROCOSMS EXPOSED TO TWO PHOSPHORUS SMOKES

Element	RP/BR Smoke		WP Smoke	
	ANOVA (by Cart x Target Dose)	Linear Regression (by P Deposition)	ANOVA (by Cart x Target Dose)	Linear Regression (by P Deposition)
Al	NSIG[a]	NSIG	NSIG	SIG[b] at .05 Level
Cr	NSIG	NSIG	NSIG ($p = .086$)	NSIG
Mo	NSIG	NSIG	NSIG	NSIG
P	SIG at .01 Level (high dose diff. from control)	SIG at .0001 Level	SIG at .01 Level (high dose diff. from control)	SIG at .0001 Level
Pb	NSIG	NSIG	NSIG	SIG at .05 Level
As	SIG at .01 Level (high dose diff. from control)[d]	SIG at .0001 Level	SIG at .05 Level (no dose differences)[e]	No Test Required[c]

(a) NSIG = nonsignificant.

(b) SIG = significant.

(c) A quadratic regression for As was significant at 0.001 level.

(d) High dose significantly different from control by Bonferroni's test.

(e) No significant dose differences by Bonferroni's test.

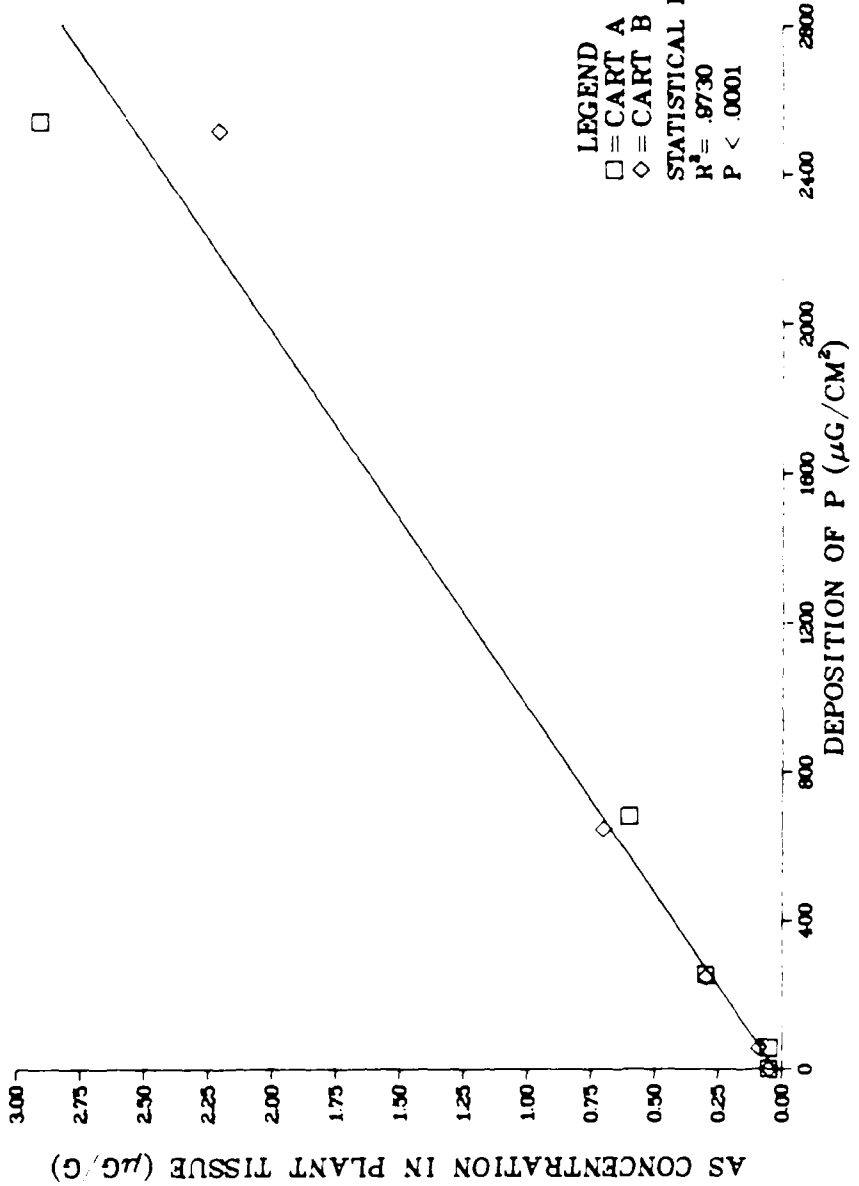


FIGURE 14. LINEAR REGRESSION OF ARSENIC CONCENTRATIONS IN PLANT TISSUE DUE TO MICROCOSM EXPOSURE TO RP/BR SMOKE VERSUS TOTAL PHOSPHORUS DEPOSITED IN THE RANGE-FINDING TEST

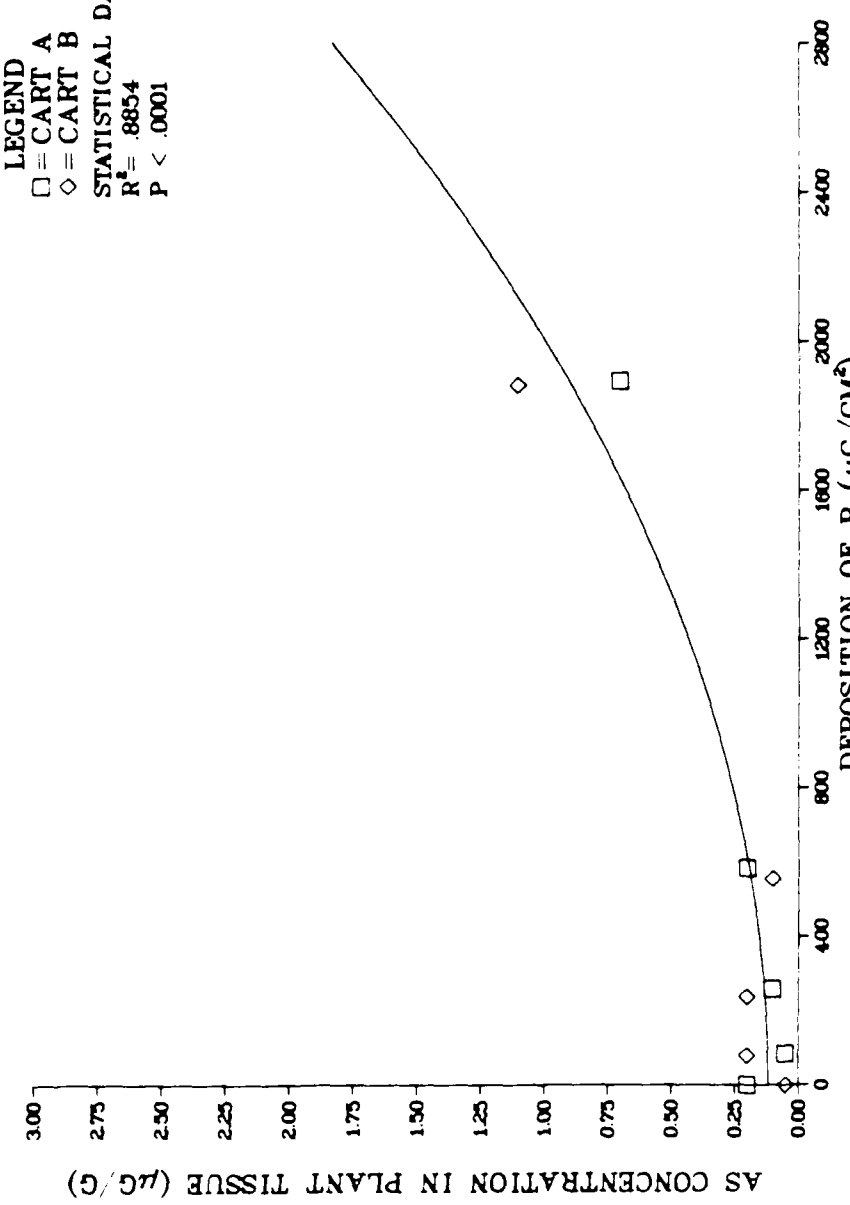


FIGURE 15. QUADRATIC REGRESSION OF ARSENIC CONCENTRATIONS IN PLANT TISSUE DUE TO MICROCOSM EXPOSURE TO WP SMOKE VERSUS TOTAL PHOSPHORUS DEPOSITED IN THE RANGE-FINDING TEST

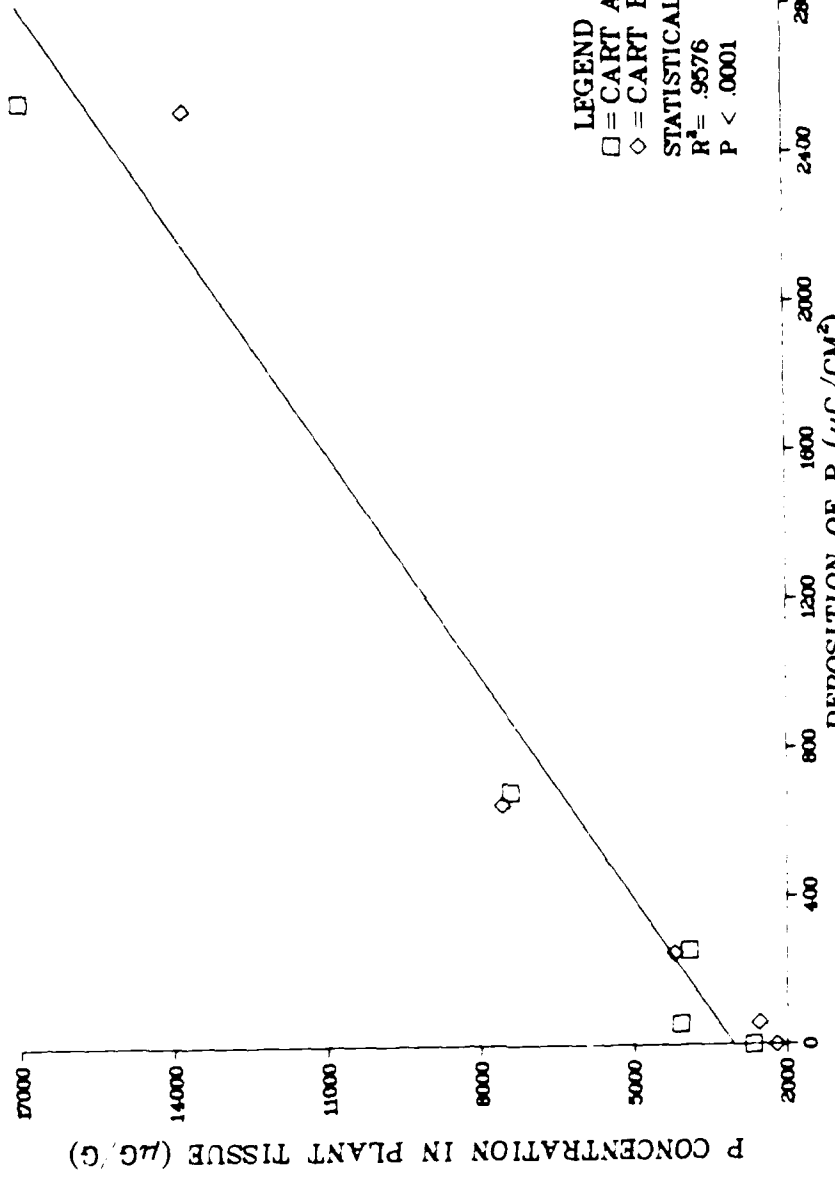


FIGURE 16. LINEAR REGRESSION OF PHOSPHORUS CONCENTRATIONS IN PLANT TISSUE DUE TO MICROCOSM EXPOSURE TO RP/BR SMOKE VERSUS TOTAL PHOSPHORUS DEPOSITED IN THE RANGE-FINDING TEST

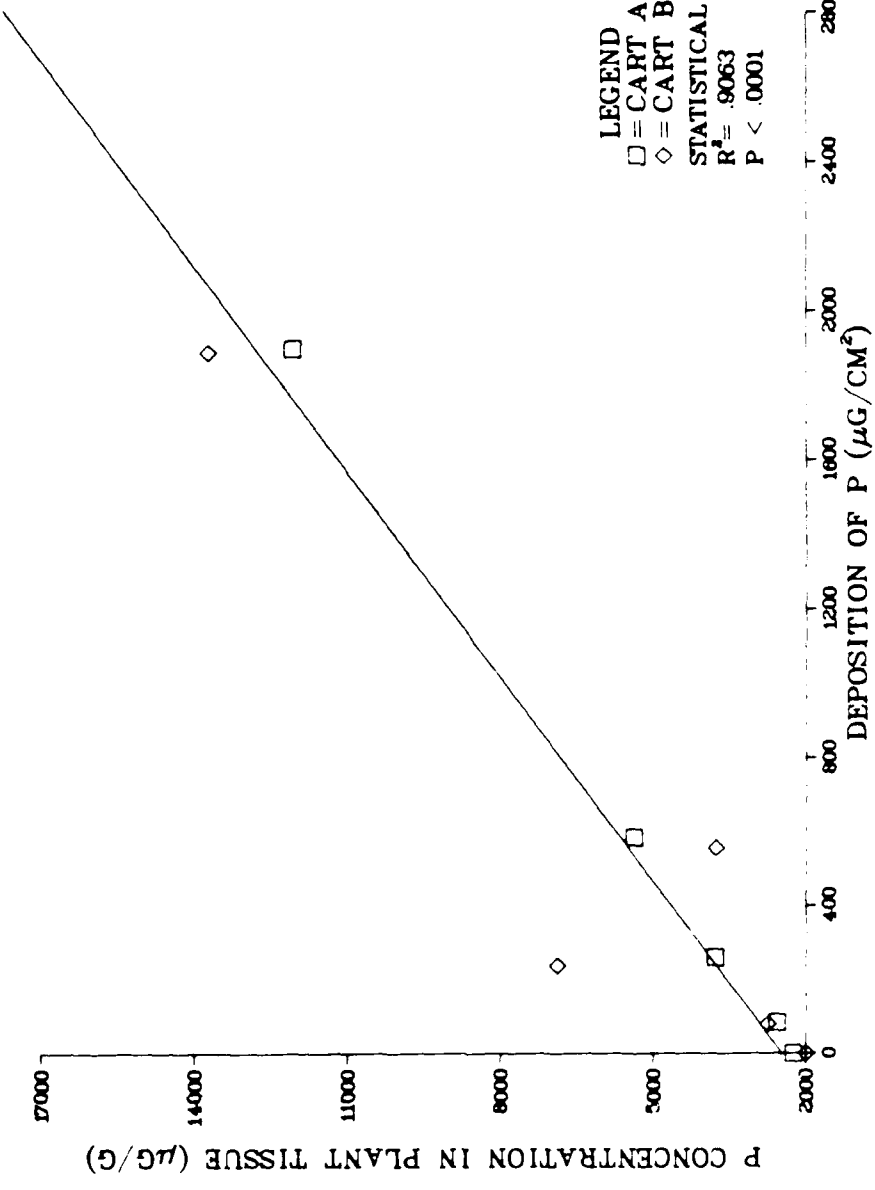


FIGURE 17. LINEAR REGRESSION OF PHOSPHORUS CONCENTRATIONS IN PLANT TISSUE DUE TO MICROCOSM EXPOSURE TO WP SMOKE VERSUS TOTAL PHOSPHORUS DEPOSITED IN THE RANGE-FINDING TEST

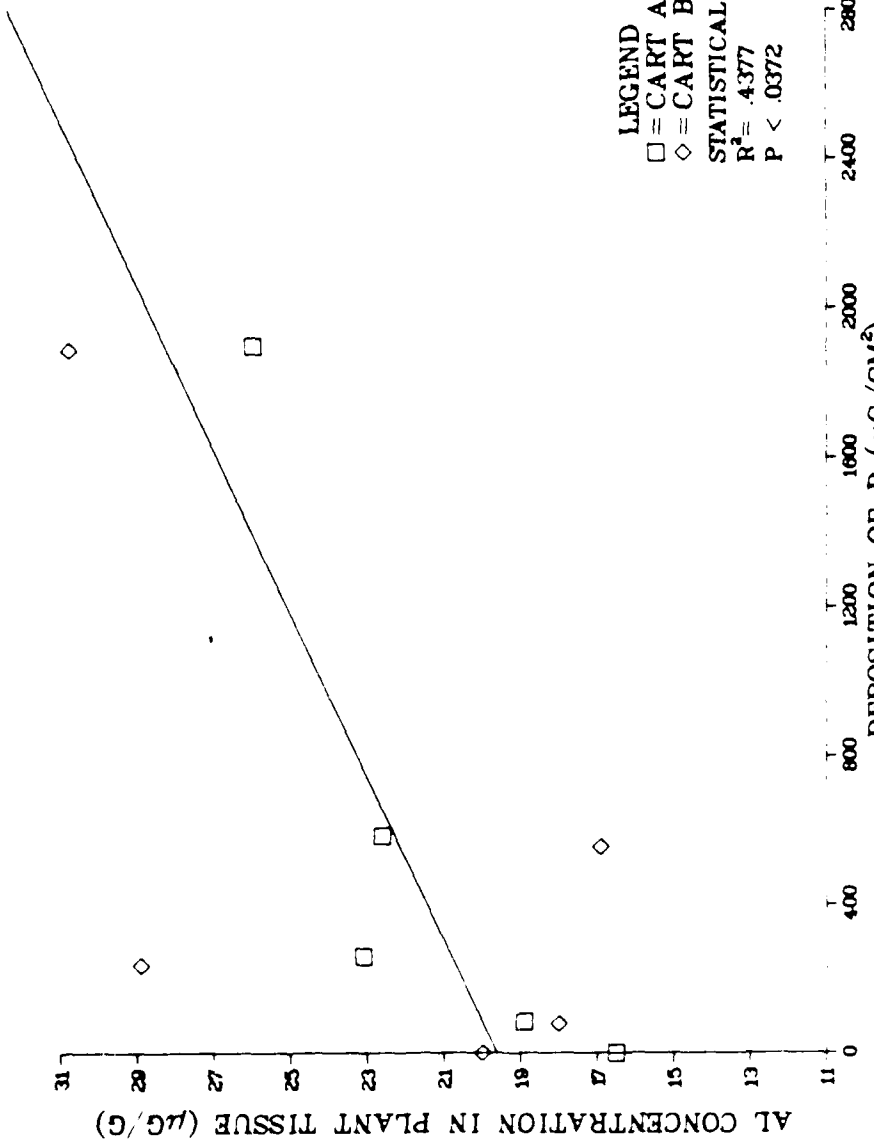


FIGURE 18. LINEAR REGRESSION OF ALUMINUM CONCENTRATIONS IN PLANT TISSUE DUE TO MICROCOSM EXPOSURE TO WP SMOKE VERSUS TOTAL PHOSPHORUS DEPOSITED IN THE RANGE-FINDING TEST

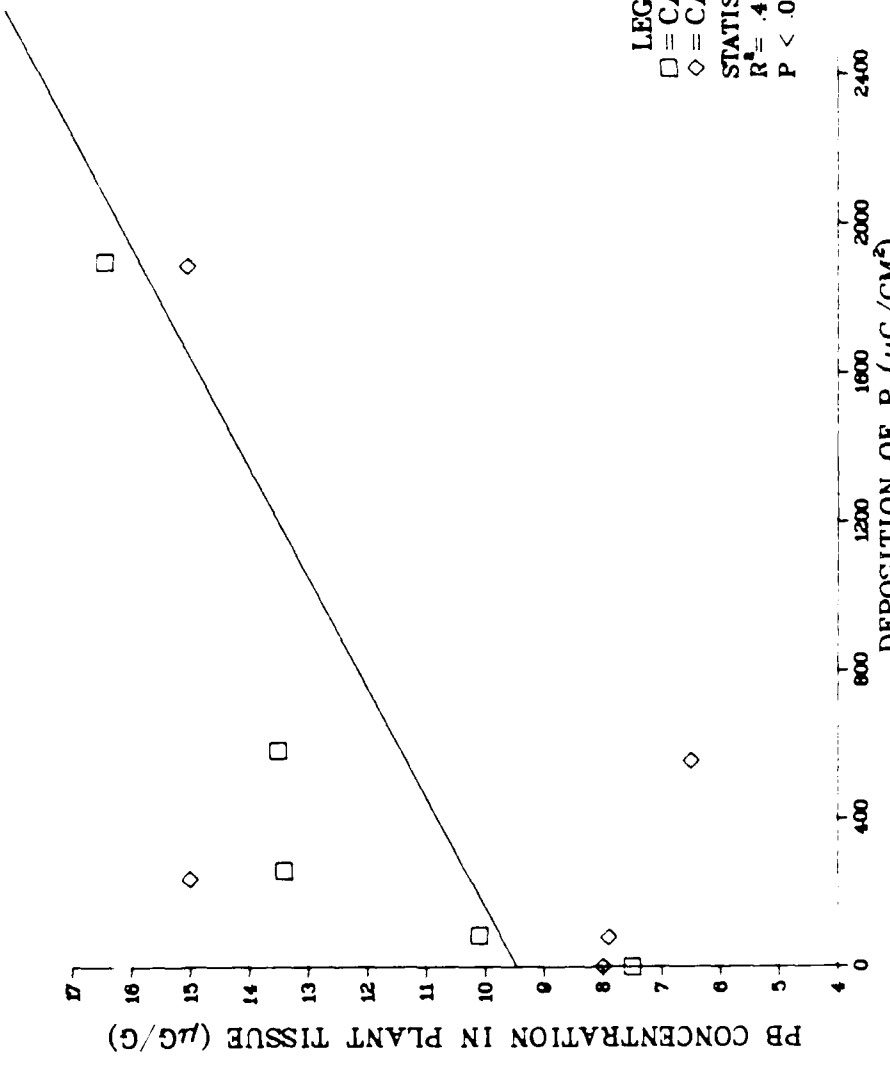


FIGURE 19. LINEAR REGRESSION OF LEAD CONCENTRATIONS IN PLANT TISSUE DUE TO MICROCOSM EXPOSURE TO WP SMOKE VERSUS TOTAL PHOSPHORUS DEPOSITED IN THE RANGE-FINDING TEST

smokes (see Table 10 and Figures 11 and 12) may be due to As, since Liebig[47] reports that legumes have low or no tolerance to this element. On the other hand, the trend toward increased wheat biomass at the high treatment level of WP smoke (see Figure 13), may be due to the beneficial effect of As on wheat. Liebig[47] reports that wheat yields were improved after application of calcium arsenate. Bonferroni's test did not detect the trend for wheat increase indicated by the quadratic regression.

The increased uptake of As and Pb does not pose a potential threat to domestic grazing animals, even at the highest treatment level. The highest mean As concentration (2.55 ppm) in plants exposed to either smoke (see Appendix B, Table B-9) is still below the level (>3.4 ppm) considered toxic to sheep. Similarly, the highest mean Pb concentration (15.7 ppm) in plants exposed to either smoke is well below the level (>80 ppm) considered toxic to horses. The threshold toxicity data for sheep and horses are used here, because they are the most sensitive grazing animals for which toxicity data are available[49].

The improved yield in sweetclover biomass at low treatment levels (see Table 10 and Figures 11 and 12) is not due to correction of a P deficiency. Plant tissue concentrations for P from this study (see Appendix B, Table B-9) are well above the levels indicated in Chapman[48] as indicative of a P deficiency. In addition, half-strength Hoagland's nutrient solution was added based on fertilizer recommendations from soil analysis (see Section 2.4.7). The total amount of phosphorus added by nutrient solution was equal to 106 kg of P_2O_5 per hectare.

Acid rain research (e.g., reference 51) and other Al toxicity research[52] indicates that Al could be toxic to terrestrial plants in acid (< pH 4.5) soils, but Al toxicity to terrestrial plants is not likely in this study. Aluminum is soluble in soils at low pH (<4.5) and high pH (>8.0). The pH of the moderate-cation-exchange-capacity (CEC) soils in this study only decreased from 7.79 in the control to 6.95 in the high treatment level (see Section 3.3.6). However, the addition of Al to terrestrial ecosystems as a result of its trace presence in the phosphorus smokes might be a potential plant toxicity problem in areas with circumneutral soils that are weakly buffered and have a low CEC. These potentially problem soils have been mapped for the U.S. by acid rain research (e.g., references 53 and 54). This study did not evaluate the potential for Al solubility in runoff and any resultant hazard to aquatic life.

Lead concentrations in high-treatment-level plants (up to 15.7 ppm; see Appendix B, Table B-9) are well above normal (0.1 to 10 ppm; reference 50) levels in uncontaminated plants. However, even the highest plant tissue levels of Pb resulting from smoke exposure are not sufficient to cause plant or animal toxicity[49,55].

In this study, the addition of As, Pb, and other elemental impurities in the phosphorus may be responsible for the significant Ca loss in leachate from RP/BR-smoke-treated microcosms (see Section 3.3.2). Jackson, et al.,[15] have shown that increasing application rates of As increased the loss of several nutrients from intact grassland microcosms. In addition, Jackson, et al.,[17] reported that forest microcosms treated with litter and baghouse dust containing Pb, Cd, Zn, and Cu showed significantly increased loss of nutrients in leachate.

3.3.5. Supplementary Studies

Two types of data were collected and analyzed that were peripheral to the main study. Leaf surface area of each species from the second harvest and light intensity during microcosm exposure were analyzed to assist in the interpretation of biomass data.

3.3.5.1 Leaf Surface Area. Analysis of leaf surface area data (see Appendix B, Table B-10) by ANOVA and the quadratic term of a regression equation indicated that only sweetclover exposed to WP smoke was significantly affected (Table 13). The quadratic term of the quadratic regression was significant ($p < 0.01$) for WP smoke. No significant effect of RP/BR smoke was detected on the leaf surface area of any of the three plant species. As expected, the quadratic regression fit to the leaf surface area data for sweetclover exposed to WP (Figure 20) is very similar to the quadratic regression fit to the biomass data for sweetclover exposed to WP (Figure 12). Since most of the leaf surfaces on sweetclover are horizontally oriented, it is possible that this resulted in more smoke aerosol deposition on sweetclover leaves than the vertically oriented leaves, even though both species had similar leaf surface areas at the control and low dose levels (see Appendix B,

TABLE 13. STATISTICAL ANALYSIS OF PLANT SURFACE AREA FROM MICROCOSMS EXPOSED TO TWO PHOSPHORUS SMOKES

Mean Surface Area	RP/BR Smoke			WP Smoke		
	ANOVA (on Target Dose)	Linear Regression[a] (on P Deposition)	Quadratic Regression (on P Deposition^2)	ANOVA (on Target Dose)	Linear Regression[a] (on P Deposition)	Quadratic Regression (on P Deposition^2)
Wheat	NSIG[b]	NSIG	NSIG	NSIG	NSIG	NSIG
Rygrass	NSIG	NSIG	NSIG	NSIG	NSIG	NSIG
Sweetclover	NSIG	NSIG	NSIG ($p = .061$)	SIG at .05 Level (None)[d]	No Test Required	SIG at .01 Level

(a) Linear regression curve was fitted when the quadratic term of the quadratic regression was not significant; otherwise, linear regression analysis was not required.

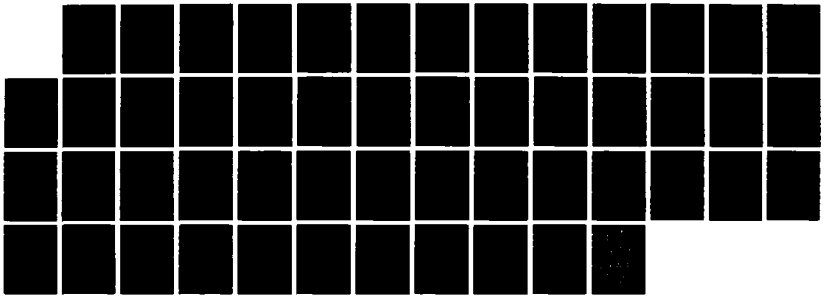
(b) NSIG = nonsignificant.

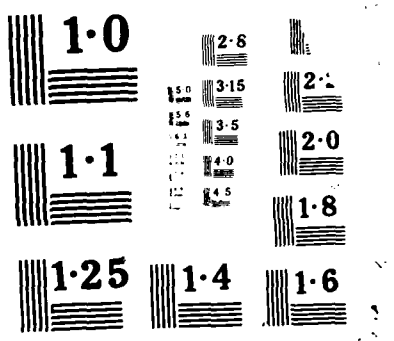
(c) SIG = significant.

(d) Dose groups significantly different from control by Bonferroni's test.

AD-A190 797 TERRESTRIAL MICROCOSM EVALUATION OF TWO ARMY SMOKE-PRODUCING COMPOUNDS(U) BATTELLE COLUMBUS DIV OH D A TOLLE ET AL. 29 JAN 88 DAMD17-84-C-4001 2/2

UNCLASSIFIED F/G 24/1 NL




1·0
1·1
1·25
1·4
1·6
1·8
2·0
2·8
3·15
3·5
4·0
4·5

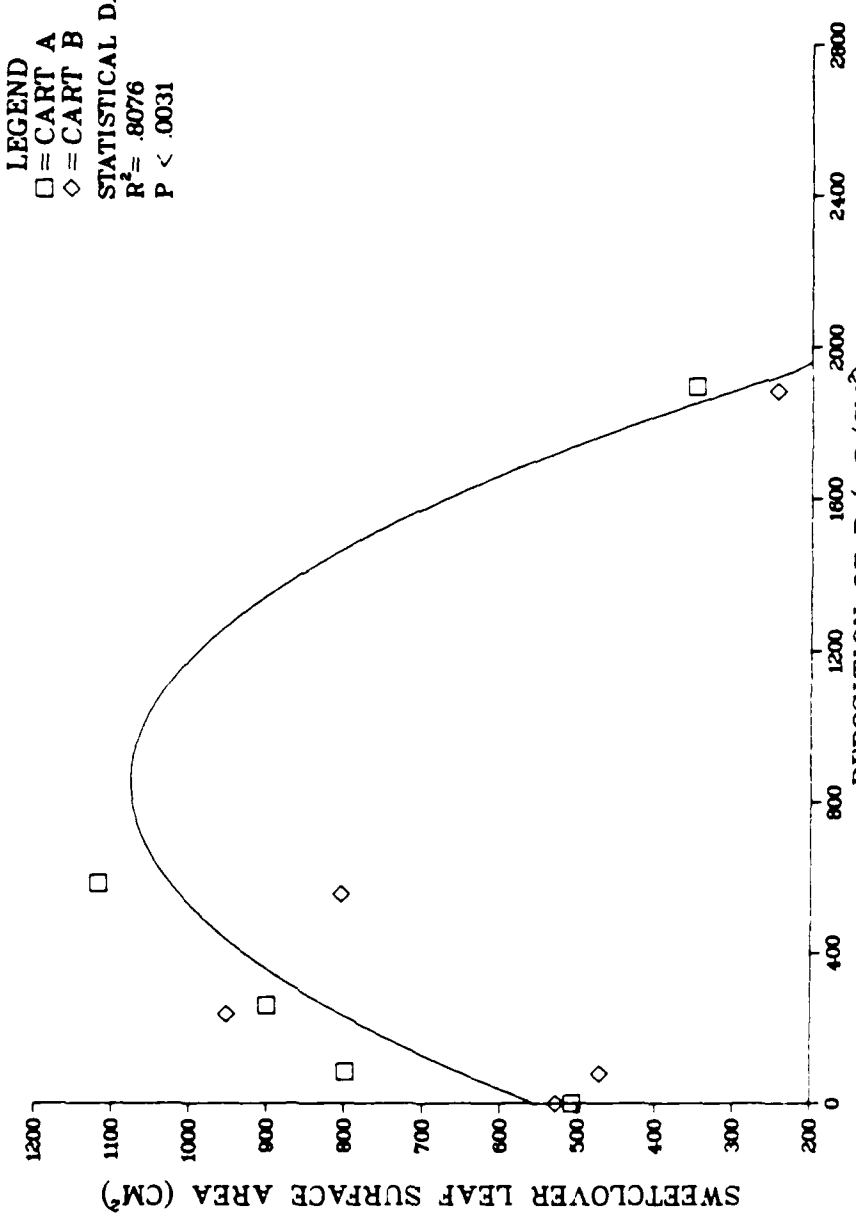


FIGURE 20. QUADRATIC REGRESSION OF WHITE PHOSPHORUS DEPOSITION VERSUS LEAF SURFACE AREA OF SWEETCLOVER IN THE RANGE-FINDING TEST

Table B-10). Thus, the horizontally oriented sweetclover leaves may have received a greater mass of smoke aerosol than the other two species, contributing to a significant biomass decline at the highest treatment level.

3.3.5.2. Light Intensity During Exposure. Light intensity data collected during smoke exposure at plant height inside the exposure chamber (see Appendix B, Table B-1) suggested that light intensity received by plants during exposure decreased as the smoke concentration increased. This effect appeared to be greatest during the first 10 minutes (during combustion). However, considerable day-to-day variability in natural light intensity occurred, making it difficult to evaluate changes inside the four exposure chambers with only one light sensor.

Light intensity data were statistically analyzed for 1-minute, 5-minute, and 10-minute time periods after lights were switched on, but no significant effects of smoke aerosol were noted on the light intensity received by plants (Table 14). The variable used was the light intensity at a given time divided by the light intensity at time 0. The linear term of a regression equation on peak P deposition levels did not detect any statistically significant effect of exposure to either smoke at any of the light measurements.

3.3.6 Soil Characterization

Post-exposure soil characterization included analysis of soil for pH, cation exchange capacity (CEC), and electrical conductivity (EC) (see Section 2.4.9). Due to the acidic nature of RP/BR and WP smokes, it was felt that pH might be the most affected of the three parameters measured. As it turned out, none of the measured parameters was affected greatly (Table 15). These data were of a supplementary nature and were not analyzed statistically. Nevertheless, the smoke exposures appeared to have little impact on the three parameters measured, with the possible exception of a slight acidifying tendency (slight drop in pH) due to increasing levels of smokes deposition. This may have been most pronounced in the case of RP/BR, in which the pH changed by approximately 0.8 pH units from the control to the highest dosed soil. In the case of WP, the pH drop was approximately 0.6 pH units from the control to the highest dosed soil. No clear pattern is evident in the remaining data to suggest any effect of the smokes on these soil parameters.

TABLE 14. STATISTICAL ANALYSIS OF LIGHT INTENSITY DATA TAKEN IN PLEXIGLAS® EXPOSURE CHAMBERS USED TO EXPOSE MICROCOSMS TO RP/BR OR WP SMOKE

Light Intensity Ratio[a]	Evaluation of the Linear Term of a Regression Equation (by Peak P deposition)	
	RP/BR Smoke	WP Smoke
Time 1/Time 0	NSIG[b]	NSIG
Time 5/Time 0	NSIG	NSIG
Time 10/Time 0	NSIG	NSIG

(a) Time periods represent the number of minutes after ignition. Light intensity data are presented in Appendix B, Table B-11.

(b) NSIG = nonsignificant.

TABLE 15. SOIL pH, CATION EXCHANGE CAPACITY (CEC), AND ELECTRICAL CONDUCTIVITY (EC) IN SOILS EXPOSED FOR 8 WEEKS TO VARIOUS TARGET CONCENTRATIONS OF RP/BR OR WP SMOKES

Smoke Target Concentration (mg/m^3)	Soil Parameter[a]		
	pH	CEC (milli-equivalents/100 g)	EC (millimhos/cm)
	Mean (±SD)	Mean (±SD)	Mean (±SD)
RP/BR			
0	7.79 (0.19)	16.6 (0.42)	1.318 (0.081)
100	8.04 (0.04)	18.4 (0.49)	1.295 (0.026)
300	7.75 (0.16)	19.3 (0.28)	1.297 (0.037)
600	7.60 (0.05)	21.0 (1.48)	1.278 (0.002)
1500	6.95 (0.06)	19.6 (2.76)	1.296 (0.031)
WP			
0	7.91 (0.30)	15.4 (0.35)	1.271 (0.011)
100	7.98 (0.01)	15.3 (0.21)	1.270 (0.013)
300	7.92 (0.04)	15.7 (0.42)	1.275 (0.011)
600	7.58 (0.08)	14.8 (1.20)	1.277 (0.004)
1500	7.35 (0.07)	27.5 (15.3)	1.266 (0.009)

(a) Values given are means ± standard deviation of two replicates. Each replicate represents three cores.

3.4 Soil Respiration Experiment

Soil microorganisms are responsible for the mineralization of organically bound (plant-unavailable) essential nutrients to the plant-available inorganic forms. Nutrients such as carbon, nitrogen, phosphorus, and sulfur are made available as the result of the metabolic activities of diverse microbial communities in the soil. Should this mineralization potential be disturbed, the ability of the soil system to support plant growth can be lost.

Accordingly, the effects of exposure of soil to RP/BR and WP smokes were evaluated to determine if soil microorganisms had been impacted. The metabolic potential of soil microorganisms was evaluated using a soil respiration test in which carbon dioxide evolution from soil was monitored (see Section 2.5). This system has proven to be a sensitive indicator of toxicity to soil microorganisms and correlates well with effects seen on higher plants[18].

Exposure of soil-core microcosms to RP/BR or WP smokes had no measurable impact on the ability of soil microorganisms to mineralize added organic substrate. Figures 21 and 22 show that microbial activity, as measured by cumulative evolution of carbon dioxide-carbon (CO_2-C) over 32 days was similar between experimental treatments. Each point in Figures 21 and 22 represents the mean of six CO_2-C values released from the soils. The rates of daily decline in CO_2-C production (b) were estimated by fitting to each cart/dose combination the model: CO_2-C production = ae^{bt}, where t is time in days and a and b are constants. The average rate for each dose is given in Table 16. The rates then were statistically analyzed by a one-way ANOVA (target dose), and a quadratic regression on target dose. When the quadratic term of the regression was not significant, a linear regression was fitted.

The ANOVA did not show any statistically significant differences between target doses of either smoke on the rate of decline of CO_2-C production. The quadratic term of a regression equation showed a statistically significant effect ($p < 0.05$) for RP/BR, but neither the quadratic nor the linear terms of regression equations showed any significant effect of WP. These data are summarized in Table 17.

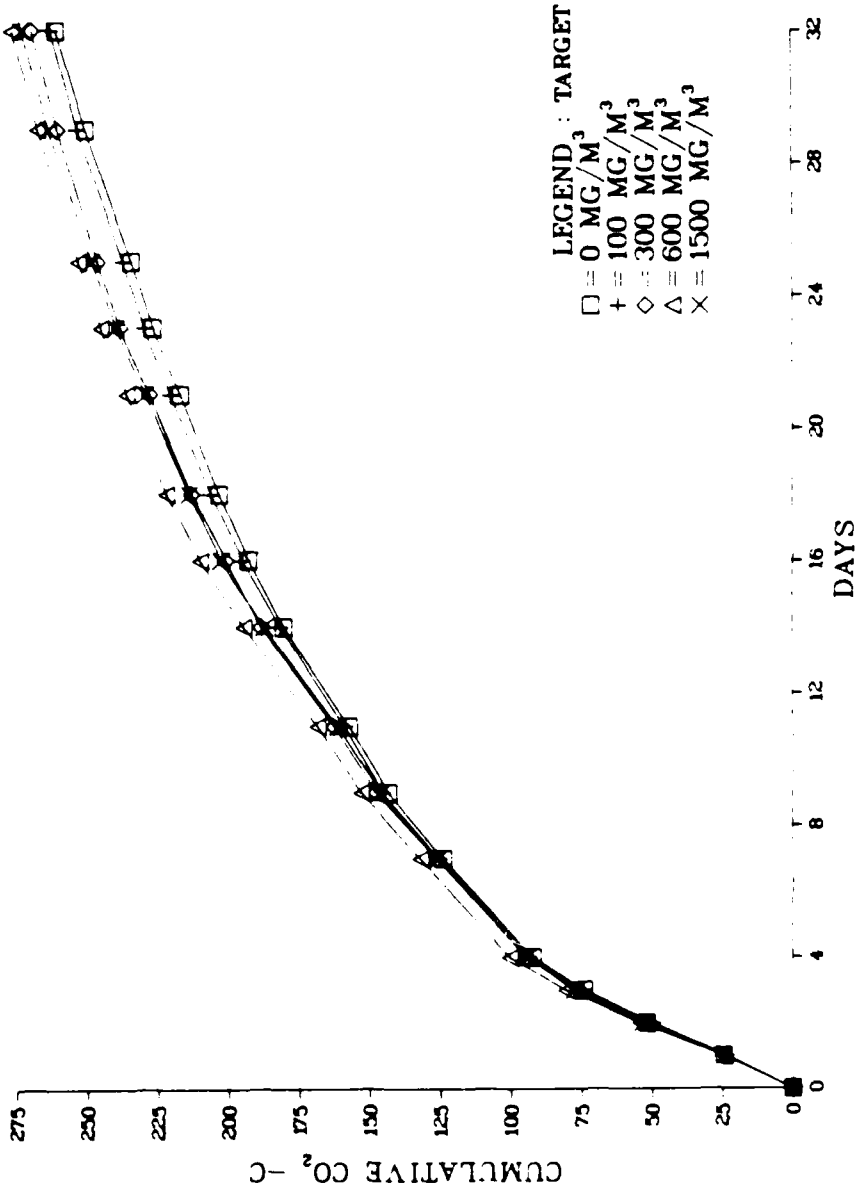


FIGURE 21. EVOLUTION OF MICROBIAL CO_2-C FROM SOILS EXPOSED FOR EIGHT WEEKS TO RP/BP SMOKE IN THE RANGE-FINDING TEST

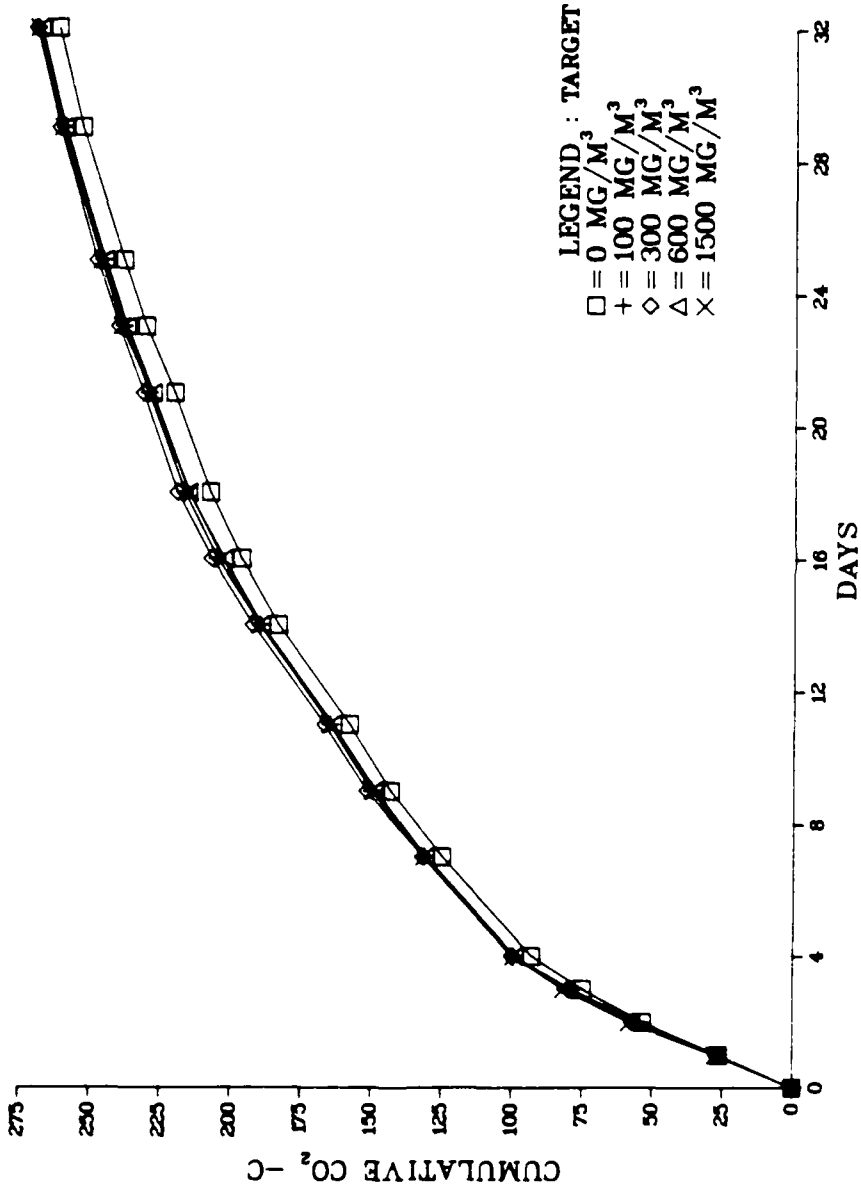


FIGURE 22. EVOLUTION OF MIRCROBIAL CO_2-C FROM SOILS EXPOSED FOR EIGHT WEEKS TO WP SMOKE IN THE RANGE-FINDING TEST

TABLE 16. INFLUENCE OF TWO PHOSPHORUS SMOKES ON CO_2-C PRODUCTION

(Values shown are the average values over two carts.)

	RP/BR Smoke					WP Smoke				
	Target Dose (mg/m^3)					Target Dose (mg/m^3)				
	0	100	300	600	1500	0	100	300	600	1500
Rate of decline[a]	-0.032	-0.034	-0.035	-0.038	-0.032	-0.035	-0.037	-0.040	-0.038	-0.038
Total CO_2-C production (mg)	259.5	261.5	268.1	275.0	271.7	260.5	268.1	268.4	266.9	267.8

(a) The rate of decline is the parameter b in the equation CO_2-C production = ae^{tb}, where t is time in days.

The results of the soil respiration experiments support the results of nutrient loss in leachates (see Section 3.3.2) and plant biomass effects (see Section 3.3.3). Very little impact on the capacity of soil microorganisms to mineralize organic material is expected due to exposure of soil to RP/BR or WP smokes. Slightly increased microbial activity may have resulted from exposure to RP/BR, especially at the target dose of 600 mg/m^3 (see Figure 21). The curves in Figures 21 and 22 resemble control (nondosed) curves for cumulative CO_2-C evolution from other toxicity experiments[18]. Although a positive control was not included in the current experiment, previous experience indicates that treatment with a highly toxic material such as 1000 ppm $CdCl_2$ would result in the cumulative release of approximately 40 mg CO_2-C over a similar time period and using a similar experimental system[12,18]. This is further substantiation that the RP/BR and WP smokes had no impact on soil microbial activity.

TABLE 17. STATISTICAL EVALUATION OF THE RATE OF DECLINE OF CO_2-C PRODUCTION OVER TIME IN SOIL RESPIRATION EXPERIMENTS

	RP/BR Smoke			WP Smoke		
	ANOVA (by target dose)	Linear[a] Regression (by target dose)	Quadratic Regression (by target dose)	ANOVA (by target dose)	Linear Regression (by target dose)	Quadratic Regression (by target dose)
Rate of decline[b] (from Equation CO_2-C Production = ae^{tb})	NSIG[b]	No Test Required	SIG[c] at .05 level	NSIG	NSIG	NSIG

(a) Linear regression curve was fitted when the quadratic term of the quadratic regression was not significant; otherwise, linear regression analysis was not required.

(b) NSIG = nonsignificant.

(c) SIG = significant.

4.0 CONCLUSIONS

4.1 Preliminary Tests

Preliminary testing, involving exposure characterization (see Section 3.1.1) and acute plant stress-ethylene response (see Section 3.1.2), was required prior to conducting the more chronic microcosm exposures. These preliminary tests were crucial to the completion of the project for two important reasons. First, the preliminary testing resulted in development of exposure methodology. During the exposure characterization it was quickly learned that combustion of smoke materials directly in exposure chambers was not only feasible but necessary. Very high concentrations of smoke inside the exposure chambers were achieved and appropriately monitored through time. Thus, the exposure characterization efforts greatly facilitated the actual exposures of microcosms.

The second reason preliminary experiments were so valuable is that the RP/BR and WP smokes were assessed as being relatively innocuous to terrestrial ecosystems, especially at expected environmental concentrations. The stress-ethylene test results showed that the actual exposures to microcosms would have to be much greater than anticipated originally. Thus, considerable effort and time was saved by gaining early experience of how the obscurant smokes would affect the soil-plant system. These acute experiments led to the design of the more chronic microcosm test and a prior knowledge of what concentrations of smokes would be necessary to elicit plant stress. Since the chronic microcosm tests involved repeat exposures, the microcosm exposures were selected below the concentrations (roughly 20,000 mg/m^3) required to elicit an acute, stress-ethylene response. In addition, the target concentrations selected for the microcosm tests bracketed typical field concentrations.

4.2 Soil Leachate Characterizations

Data obtained from the leaching of soil columns indicate that phosphate aerosols deposited at a rate of up to 20,000 mg/m^3 do not influence the levels of dissolved K, Ca, NH_4-N, NO_3-N, and TOC. These results indicate that

the soil nutrient buffering capacity represented by an approximate 15-cm soil depth is not affected by the level of acidity applied at this level of aerosol concentration. Thus, any significant nutrient loss from the microcosms (e.g., Ca) associated with the application of phosphate aerosols may be attributable to biologically mediated responses and not to effects on physicochemical soil processes.

4.3 Microcosm Test

Although the Results and Discussion Section (3.0) of this report described the results from both phosphorus smokes together, the ecosystem effects conclusions of the range-finding test are described individually below for each smoke. The four ecosystem effects (i.e., nutrient loss, biomass, element uptake, and soil respiration) are discussed separately for RP/BR (Section 4.3.2) and WP (Section 4.3.3.). Conclusions about the exposure characterization for both smokes are discussed together.

4.3.1 Exposure Characterization

The characterization of the exposures required measurement of the temperature, relative humidity, and dosage for each test. These measurements were obtained for each test, and limited measurements were made to insure that other products of combustion which might be physiologically significant were not present--which they were not. The precision of this technique for deposited phosphorus determination is limited, especially at the lower exposure levels where the relative standard deviation in the measurements is on the order of $\pm$ 30 percent. This value is more than halved at the higher exposure levels. Clearly, this technique of exposure measurement would require refinement if small differences in the exposure levels resulted in significant changes in the measured response. But it was recognized at the outset that large differences in exposures were likely to be required to elicit significantly different responses, so this technique was deemed appropriate. Analyses of variance were performed and demonstrated that all four of the exposure levels were significantly different from one another at the $p < 0.0001$ level for all but the two low exposures (100 and 300 mg/m^3) for the WP. T-tests

were performed and demonstrated that in no case was there a significant difference between the A and B groups for any exposure level. Judging from these reuslts, it appears that the method of exposure used was capable of generating the required exposure levels, although not as precisely as one would desire for more toxic materials.

One additional measurement would be useful in testing such as this, although it is recognized that it is a difficult measurement to perform routinely. This is the measurement of the plant (and soil) surface area actually exposed to the smoke. While measurements of biomass or total leaf surface area can be made at the end of a set of exposures, there does not appear to be a simple means available for measuring the upward facing (or even total) surface area in a nondestructive manner. The lack of such a measurement could mask a significant source of variability in the measured responses to identical exposures under some conditions.

4.3.2 Ecosystem Effects of RP/BR Smoke

The ecosystem effects of RP/BR smoke were evalauted by monitoring four parameters in terrestrial microcosms. These parameters included nutrient loss in soil leachate, biomass yield, element uptake in plants, and soil microorganism respiration. As discussed in the following subsections, only minor effects were noted in the first three parameters at the highest (1500 mg/m^3) treatment level, but this concentration is far above expected field levels. No negative ecosystem effects were observed at the three lower concentrations of RP/BR.

4.3.2.1 Nutrient Loss in Soil Leachate. Exposure of planted soil-core microcosms to various concentrations of RP/BR smokes had little effect on nutrient loss in soil. The only significant effect due to RP/BR was on cumulative loss of Ca. Data (see Figure 10) indicate a greater cumulative loss of Ca at the highest deposition of phosphorus, compared to other deposition levels.

The significance of this finding is not clear because other nutrients apparently were not affected. In the work of Likens et al.[32], increased loss of Ca in soil water from a clear-cut forested watershed was

attributed largely to displacement of Ca from soil exchange sites by hydrogen ions. The hydrogen ion production was attributed to increased nitrification and concomitant increased loss of nitrate in groundwater. In the current study, increased nitrate loss due to RP/BR exposure was not observed. However, slight soil acidification was noted (see Section 3.3.6), implying a potential for increased flux of hydrogen ions to displace Ca. Because soil leaching studies showed no such Ca displacement by H^+, a possible explanation for increased Ca loss at high exposures to RP/BR is a reduction in sweetclover biomass yield (Section 4.3.3.2). Legumes generally contain about 1.4 percent Ca by dry weight while grasses contain approximately 0.3 percent Ca by dry weight. While no Ca deficiencies in plant tissue were noted, the reduced sweetclover growth at high exposures would require less Ca uptake, leading potentially to greater losses in soil water.

The major conclusion is that exposure to RP/BR, even at relatively high concentrations, had little impact on nutrient losses in microcosm leachates. These results suggest that microcosm plants were not affected to any great extent due to RP/BR smoke, and this conclusion is generally supported by biomass yield data (see Section 3.3.3). The effects that were observed occurred only after repeated exposures to a level of RP/BR (1,500 mg/m^3) that is much greater than expected environmental exposures.

4.3.2.2 Biomass Yield. The combined biomass (all three species for two harvests) yield from microcosms was not significantly affected by 16 semi-weekly exposures to RP/BR smoke, even at levels (1500 mg/m^3) far above expected field concentrations. However, a significant ($p < 0.05$) decline in biomass occurred for sweetclover, based on regression analyses of the individual species dry weights from the second harvest. It is possible that at the highest treatment level (1500 mg/m^3) the As added to the soil as a contaminant in the RP may have caused the decline of sweetclover, because legumes are very intolerant of As[47]. It is also possible that the large surface area and horizontal orientation of sweetclover leaves resulted in a greater mass of smoke aerosol being deposited on the leaves, thus exposing the sweetclover to a larger effective dose compared to the other two species.

Since realistic field exposures of RP/BR smoke are expected to be far below the high (1500 mg/m^3) dose level used in this study, no effects on plant biomass are predicted from normal training activities using RP/BR smoke.

No trend toward increasing wheat biomass at the highest treatment level was detected for RP/BR, even though this trend was determined by regression analysis for WP. Calcium arsenate has been shown to increase wheat biomass, when applied at low levels[47]. Plants in control microcosms had As concentrations below the 0.15 to 0.30 ppm considered intermediate between deficiency and toxicity for wheat. Thus, it is possible that the As levels in plant tissue (2.55 ppm) at the highest RP/BR treatment level were beyond the stimulatory stage for wheat and beginning to reach toxic levels, while As concentrations in plant tissue (0.9 ppm) at the highest WP smoke treatment level were still in the stimulatory range for wheat.

4.3.2.3 Element Uptake by Plants. Element uptake data did not show any significant negative effects, except at RP/BR smoke concentrations of 1500 mg/m^3. Of the two elements (P and As) where significant uptake in plants was noted, only As had the potential for negative impact on biomass or nutrient loss at the high treatment level. Arsenic added to the soil as an impurity in the RP may be present in sufficient concentration to cause the biomass decline in sweetclover, because legumes are very intolerant of As[47]. The addition of As to the soil may also be responsible for the significant increase in Ca loss in leachate from RP/BR-smoke-treated microcosms, since toxic element application to terrestrial microcosms in other studies has resulted in increased nutrient loss[15,17,31]. None of the element uptake data indicated any potential impact on domestic grazing animals, because toxic element concentrations in plant tissue were below the threshold concentrations for the most sensitive domestic grazing animals for which toxicity data were available.

4.3.2.4 Soil Microorganism Respiration. Exposure of planted soil-core microcosms to various levels of RP/BR smoke had no impact on respiration of soil microorganisms, as measured by release of CO_2-C from alfalfa meal-amended soils. Although no single assay is capable of monitoring the metabolic activities of all soil microorganisms, the soil respiration assay has the advantage that a very large proportion of soil microbial activity is

detected. The assay as performed in the current project has substantial environmental relevance because the mineralization of a natural substrate with a narrow carbon-to-nitrogen ratio, finely ground alfalfa meal, is used to assay the activity of diverse soil heterotrophic microorganisms. The results compare well with other observations on nutrient loss and biomass yield and support the conclusion that RP/BR smoke, especially at expected environmental concentrations, has little detrimental impact on the soil-plant ecosystem.

4.3.3 Ecosystem Effects of WP Smoke

The ecosystem effects of WP smoke were evaluated by monitoring four parameters in terrestrial microcosms, including nutrient loss in soil leachate, biomass yield, element uptake in plants, and soil microorganism respiration. As discussed in the following subsections, only minor effects were noted on biomass yield and element uptake at the highest (1500 mg/m^3) treatment level, but this concentration is far above expected field levels. No negative ecosystem effects were observed at the three lower concentrations of WP.

4.3.3.1 Nutrient Loss in Soil Leachate. Nutrient losses in soil leachate were not affected by exposure to WP smoke (see Section 3.3.2). Whereas exposure to relatively high levels of RP/BR resulted in increased loss of Ca, no such effects were noted due to WP smoke. These results coincide with the results from the biomass yield experiments (see Section 3.3.3) and the soil respiration experiments (see Section 3.4). Thus, little if any impact on the soil-plant system is expected due to exposure to WP smoke.

4.3.3.2 Biomass Yield. The combined biomass (all three species for two harvests) yield from microcosms was not significantly affected by 16 semi-weekly exposures to WP smoke, even at levels (1500 mg/m^3) far above expected field concentrations. However, significant ($p < 0.05$) effects on individual species biomass for the second harvest due to exposure to WP smoke were detected by analysis of both ANOVA and the linear or quadratic terms of regression equations for wheat and sweetclover. The wheat showed a regression trend to increase above controls at the high treatment level, but this was not

detected by Bonferroni's test. The sweetclover was significantly greater than controls at the middle dose and significantly less than controls at the high dose, based on Bonferroni's test.

It is possible that the opposite effects on wheat and sweetclover at the 1500 mg/m^3 dose may both be the result of As added to the soil as a contaminant in the WP. Legumes, such as sweetclover, are very intolerant of As, while wheat yield has been improved in other studies by application of calcium arsenate(47). Also, the large surface area and horizontal orientation of sweetclover leaves may have resulted in a greater mass of smoke aerosol deposited on the leaves of this species compared to the other two species, which caused more effects of the deposited aerosol on sweetclover. Thus, the decline in sweetclover biomass and the increase in wheat biomass at the high treatment level of WP smoke resulted in no net effect when the total biomass was analyzed. This phenomenon could potentially result in species shifts in certain ecosystems, but only at WP smoke concentrations far above those expected in the field.

Since realistic field exposures of WP smoke are expected to be far below the high (1500 mg/m^3) dose level used in this study, no effects on plant biomass are predicted from normal training activities using WP smoke.

4.3.3.3 Element Uptake by Plants. Element uptake data did not show any significant negative effects, except at WP smoke concentrations (1500 mg/m^3) far above those expected during field exercises. Of the four elements (P, Al, As, and Pb) where effects on element uptake were determined statistically, only As had the potential to cause a negative impact on biomass at the high treatment level. The addition of As to the soil as an impurity in the WP may have caused the biomass decline in sweetclover, because legumes are very intolerant of As(47). None of the element uptake data indicated any potential impact on grazing animals due to toxic element concentrations in plant tissue.

4.3.3.4 Soil Microorganism Respiration. No effect on soil microbial activity, as measured by the mineralization of added organic matter (finely grown alfalfa meal), was observed as a result of exposure of soils to various concentrations of WP smoke. After eight weeks of exposure to WP

smoke, soil heterotrophic microbial activity was unaffected, as compared to control (undosed) soils and to previous experiments[18]. These results compare well with results from experiments on nutrient loss and biomass yield and lead to the conclusion that WP smoke has little impact on the soil-plant system.

5.0 SUMMARY

Based on the results of the microcosm evaluation for phosphorus smokes, recommendations are made in the following three areas: (1) utility of the microcosm technique for testing additional types of aerosols, (2) use of the expanded range-finding test as a less expensive substitute for the more traditional range-finding plus definite type of testing, and (3) implications for the use of RP/BR or WP smoke on Army training grounds.

The recommendations made here are based on an expanded range-finding test which: (1) involved a series of repeated static exposures, and thus, did not simulate horizontal, wind-driven impacts on plants, including smoke particle impact on the down side of leaves as might occur in the field, (2) used only one type of soil, (3) used grasses and legumes (no trees), (4) simulated only one geographic area, and (5) was not designed to evaluate the potential runoff effects due to acid solubilization of metals.

5.1 Utility of the Microcosm Technique for Testing Aerosols

The terrestrial microcosm and static expsoure chamber system (see Figure 1) used in this study produced positive dose-response curves for ecological effects (see Section 3.3) with two low toxicity aerosols (RP/BR and WP smoke). Thus, the system appears to be appropriate for evaluating the static, long-term, particle-deposition effects of other aerosols. However, the dynamic, short-term, wind-driven effects on plant leaves were not evaluated in this study. The soil-core microcosm approach could be combined with a dynamic exposure chamber to evaluate wind-driven effects on the exposed (plant tops) portion of the microcosm. Significant effects of exposure to either RP/BR or WP were found for nutrient (Ca) loss, biomass (wheat and sweetclover), and element (Al, As, and Pb) uptake by plants. Dose-response curves were fit to these effects data using either a quadratic or linear model. These effects were adequately explained by physical, chemical, and biological information in the scientific literature. The ecosystem effects of RP/BR and WP smoke observed in the microcosms, however, were elicited only at smoke exposure levels far above those expected during field training exercises. Effects might have been elicited at lower exposure levels if the microcosm were combined with a dynamic exposure system.

The terrestrial microcosm technique used in this study is particularly relevant to hazard assessment. First, this laboratory technique has been shown to accurately predict effects recorded from equivalent treatment of a waste material applied in the field[12]. Second, it consists of numerous species of plants and soil organisms in an intact soil column, and thus, duplicates the physical, chemical, and biological processes that occur in the field more accurately than single species studies. Compensatory mechanisms that occur in terrestrial ecosystems among and between the biotic and abiotic components occur naturally in this system. Third, the terrestrial microcosm design used in this study has been shown to accurately predict effects over periods as long as two years[12], and thus can be continued for long-term chronic studies if preliminary data suggest a potential problem.

5.2 Expanded Range-Finding Test as Substitute for Range + Definitive Testing

The expanded range-finding test used in this study adequately produces input for hazard assessment at a considerable savings in cost and time compared to the more traditional range-finding plus definitive test strategy. The preliminary, plant stress-ethylene test essentially replaces a microcosm range-finding test and the expanded microcosm range-finding test combines aspects of both the traditional range-finding and definitive microcosm tests. The plant stress-ethylene test recommended by the U.S. Environmental Protection Agency (U.S. EPA) [22,,23] provides information on the appropriate dose range at less than 20 percent of the cost and time required for a typical, 4-week range-finding test with microcosms[12]. Similarly, the expanded range-finding test used in this study provided adequate information on four ecosystem effects parameters at less than 50 percent of the cost and time required for a full-growing-season definitive test[12].

The expanded range-finding test was designed (see Section 2.1.1) to initially evaluate plant uptake of a large number of elements (24) and loss in leachate of a large number of nutrients (5) during early stages of the test. Based on the first set of analyses, these numbers were reduced to a smaller set of indicator elements and nutrients, which were monitored during the remainder of the test. This procedure permitted screening of potential effects on a large number of elements and nutrients without the high cost of analysis on all elements and nutrients for the entire test. Only those elements and nutrients which showed a potential for impact were monitored for the full 12-week period.

The expanded range-finding approach used in this study is similar to that adopted by the U.S. EPA for their current Level 1 environmental assessment biological tests[22]. An earlier version of the Level 1 protocols included both range-finding and definitive testing[56]. The range-finding test used three to five widely-spaced test material concentrations in order to determine the concentrations that would be used in the definitive test. The range-finding and definitive tests were similar except for the spacing of the concentrations and number of replicates. The more recent Level 1 test protocols, however, combine aspects of both types of tests into a single, expanded range-finding test. This merging of two tests into one has resulted in excellent hazard assessment data at a considerable reduction in time and cost.

5.3 Implications for Smoke Use on Training Grounds

Static exposures of both the RP/BR and WP/F smoke appear to cause no significant negative ecological effects on terrestrial ecosystems at concentrations equal to or less than 600 mg/m^3, even for semiweekly exposures over a 4-month period. Since typical field concentrations and number of repeat exposures at a single site are below this level for both smokes, no impacts on terrestrial ecosystems are expected. Training ground managers can continue to deploy RP/BR or WP/F smokes at or below the above concentration and frequency without significant problems to most terrestrial systems. It should be cautioned that the static exposures used in this study were designed to mimic the short-term passage of peak smoke concentrations over vegetation, under very low wind. Continuous smoke generation, where vegetation is exposed to peak smoke concentrations lasting for more than 10 minutes, or smoke impacts under windy conditions were not evaluated.

One or a combination of three situations could cause minor negative impact by phosphorus smokes on training grounds. First, if the single, acute smoke concentration exceeds 20,000 mg/m^3, sensitive plant species, such as sweetclover may be directly affected. Second, if the chronic, repeated smoke concentrations reach or exceed 1,500 mg/m^3 and/or the frequency of exposure dramatically exceeds 16 exposures in 2 months, negative ecosystem effects may include: (1) increased Ca loss from the soil's nutrient pool to groundwater, (2) decreased biomass of sensitive species (e.g., legumes) resulting in

species shifts to more tolerant species, and (3) increased plant uptake of elements potentially toxic to plants or animals (e.g., As, Pb, and Al). In addition, if these high smoke concentrations and exposure frequencies are used on training grounds with noncalcareous, low-cation-exchange-capacity, circumneutral soils, the resulting drop to an acid soil pH will make potentially toxic elements more available to plants, especially Al. The soils considered potentially sensitive to acid precipitation (and thus to acidic aerosols of WP/F or RP/BR smoke) have been mapped by acid rain research (e.g., references 53, 54).

6.0 LITERATURE CITED

(1) Ballou, J. E. 1981. Chemical characterization and toxicologic evaluation of airborne mixtures. AD No. A102678. Contract No. DAMD17-79-C-9160. U.S. Army Medical Research and Development Command, Fort Detrick, Frederick MD.

(2) Wasti, K., K.J.R. Abaidoo, J. E. Villaume, and P. N. Craig. 1978. A literature review--problem definition studies on selected toxic chemicals. Volume 2 of 8: Occupational health and safety aspects of phosphorus smoke compounds. AD No. A056019. Contract No. DAMD17-77-C-7020. U.S. Army Medical Research and Development Command, Fort Detrick, Frederick, MD.

(3) Katz, S., A. Snelson, R. Butler, W. Bock, N. Rajendran, and S. Relwani. 1981. Physical and chemical characterization of military smokes: Part III - White phosphorus-felt smokes. AD No. A115657. Contract No. DAMD17-78-C-8085. U.S. Army Medical Research and Development Command, Fort Detrick, Frederick, MD.

(4) Burton, F. G., M. L. Clark, R. A. Miller, and R. E. Schirmer. 1982. Generation and characterization of red phosphorus smoke aerosols for inhalation exposure of laboratory animals. Am. Ind. Hygiene Assoc. J. 43:767-772.

(5) Giesy, J. P., Jr., ed. 1980. Microcosms in ecological research. CONF 781101. Technical Information Center, U.S. Department of Energy, Washington, D.C., 1,110 pp.

(6) Gillett, J. W., and J. M. Witt, eds. 1979. Terrestrial microcosms. The procedeedings of the workshop on terrestrial microcosms. NSF/RA 79-0027. National Science Foundation, Washington, D.C., 35 pp.

(7) Hammons, A. S. 1981. Methods for ecological toxicity -- a critical review of laboratory multispecies tests. Ann Arbor Science Publishers, Inc., Ann Arbor, MI., 310 pp.

(8) Tolle, D. A., M. F. Arthur, J. Chesson, and P. Van Voris. 1985. Comparison of pots versus microcosms for predicting agroecosystem effects due to waste amendment. Environ. Tox. Chem. 4:501-509.

(9) Van Voris, P., and D. A. Tolle. 1979. Field and microcosm investigation of the effects of atmospheric deposition from fossil fuel combustion. First Annual Progress Report RP1224-5 to Electric Power Research Institute, Palo Alto, CA. Battelle's Columbus Laboratories, Columbus, OH.

(10) Tolle, D. A., P. Van Voris, M. F. Arthur, J. P. Morris, and M. Larson. 1981. Evaluation of terrestrial microcosms for predicting ecosystem response to perturbation (Abstract). Bull. Ecol. Soc. America 62:141-142.

(11) Van Voris, P., D. A. Tolle, and M. F. Arthur. 1981. Fly ash utilization in the United States: environmental considerations. Pages 389-404. In: Proceedings of the First International Waste Recycling Symposium, November 16-18, Clean Japan Center, Tokyo, Japan.

(12) Van Voris, P., D. A. Tolle, M. F. Arthur, J. Chesson, and T. C. Zwick. 1984. Development and validation of a terrestrial microcosm test system for assessing ecological effects of utility wastes. EPRI EA-3672, Electric Power Research Institute, Palo Alto, CA.

(13) Zwick, T. C., M. F. Arthur, D. A. Tolle, and P. Van Voris. 1984. A unique laboratory method for evaluating agro-ecosystem effects of an industrial waste product. Plant Soil. 77:395-399.

(14) O'Neill, R. V., B. S. Ausmus, D. R. Jackson, R. I. VanHook, P. Van Voris, C. Washburne, and A. P. Watson. 1977. Monitoring terrestrial ecosystems by analysis of nutrient export. Water Air Soil Pollut. 8:271-277.

(15) Jackson, D. R., B. S. Ausmus, and M. Levin. 1979. Effects of arsenic on nutrient dynamics of grassland microcosms and field plots. Water Air Soil Pollut. 11:13-21.

(16) Tolle, D. A., M. F. Arthur, and P. Van Voris. 1982. Evaluation of terrestrial microcosms for environmental studies of utility wastes. Third Annual Progress Report on RP 1224-5 to Electric Power Research Institute, Palo Alto, CA. Battelle's Columbus Laboratories, Columbus, OH.

(17) Jackson, D. R., W. J. Selvidge, and B. S. Ausmus. 1978. Behavior of heavy metals in forest microcosms: II. Effects on nutrient cycling processes. Water Air Soil Pollut. 10:13-18.

(18) Arthur, M. F., T. C. Zwick, D. A. Tolle, and P. Van Voris. 1984. Effects of fly ash on microbial CO_2 evolution from an agricultural soil. Water Air Soil Pollut. 22:209-216.

(19) Abeles, F. B. 1972. Biosynthesis and mechanisms of action of ethylene. Ann. Rev. Plant Physiol. 23:259-292.

(20) Tingey, D. T., C. Standley, and R. W. Field. 1976. Stress Ethylene evolution: a measure of ozone effects on plants. Atmospheric Environment 10:969-974.

(21) Hurlbert, S. H. 1984. Pseudoreplication and the design of ecological field experiments. Ecol. Monogr. 54:187-211.

(22) Brusick, D. J., and R. R. Young. 1981. IERL-RTP procedures manual: Level 1 environmental assessment biological tests. EPA-600 8-81-024. Industrial Environmental Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC.

(23) Thompson, C. R., G. Kats, P. Dawson, and D. Doyle. 1981. Project Summary: Development of a protocol for testing effects of toxic substances on plants. EPA-600/S3-81-006. Center for Environmental Research Information, Cincinnati, OH.

(24) Jackson, D. R., B. C. Garrett, and T. A. Bishop. 1984. Comparison of Batch and Column Methods for Assessing Leachability of Hazardous Waste. Environ. Sci. and Technol. 18:668-673.

(25) U.S. Army Dugway Proving Ground. 1977. Methodology investigation for testing effectiveness of smoke/aerosol munitions: pilot study. TECOM Proj. No. 7-CO-RD6-DPI-005. DPG Document No. DPG-FR-76-701. U.S. Army Test and Evaluation Commnand, Attn: DRSTE-ME, Aberdeen Proving Ground, MD.

(26) Sem, G. J., and K. Tsurubayashi. 1975. A new mass sensor for respirable dust measurements. Am. Ind. Hygiene Assoc. J. 36:791-796.

(27) U.S. Department of the Interior. 1969. FWPCA Method for Chemical Analysis of Water and Wastes: The Determination of Phosphorus. Analytical Quality Control Laboratory, Division of Water Quality Research, Federal Water Pollution Control Administration, Cincinnati, OH.

(28) Tolle, D. A., M. F. Arthur, and P. Van Voris. 1983. Microcosm/field comparison of trace element uptake in crops grown in fly ash-amended soil. Sci. Tot. Environ. 31:243-261.

(29) Van Voris, P., M. F. Arthur, and D. A. Tolle. 1982. Evaluation of terrestrial microcosms for assessing ecological effects of utility wastes. EPRI EA-2364. Electric Power Research Institute, Palo Alto, CA.

(30) Ohio Cooperative Extension Service. 1981. Agronomy Guide. The Ohio State University, Columbus, OH. 97 pp.

(31) Van Voris, P., R. V. O'Neill, W. R. Emanuel, and H. H. Shugart, Jr., 1980. Functional complexity and ecosystem stability. Ecology 61:1352-1360.

(32) Likens, G. E., F. H. Bormann, N. M. Johnson, D. W. Fisher, and R. S. Pierce. 1970. Effects of forest cutting and herbicide treatment on nutrient budgets in the Hubbard Brook watershed-ecosystem. Ecol. Monogr. 40:23-47.

(33) Bormann, F. H., G. E. Likens, T. G. Siccama, R. S. Pierce, and J. S. Eaton. 1974. The export of nutrients and recovery of stable conditions following deforestation of Hubbard Brook. Ecol. Monogr. 44:255-277.

(34) McLean, E. O. 1980. Recommended pH and lime requirement tests, pp. 5-8. In: Recommended Chemical Soil Test Procedures for the North Central Region, North Central Regional Publication No. 221 (Revised). North Dakota Agricultural Experiment Station, North Dakota State University, Fargo, ND.

(35) Watson, M. E. 1978. Soil Testing Procedures, Research Extension Analytical Laboratory. Ohio Agricultural Research and Development Center, Wooster, OH. 20 pp.

(36) Allen, S. E., H. M. Grimshaw, J. A. Parkinson, and C. Quarmly. 1974. Chemical Analysis of Ecological Materials. Wiley and Sons, New York. 565 pp.

(37) SAS Institute, Inc. 1982. SAS User's Guide 1982 Edition. SAS Institute, Inc., Cary, NC.

(38) Neter, J., and W. Wasserman. 1974. Applied linear statistical models. Richard D. Irwin, Inc., Homewood, IL.

(39) Draper, N. R., and H. Smith. 1981. Applied regression analysis. John Wiley & Sons, Inc., New York, NY.

(40) Taylor, O. C., C. R. Thompson, D. T. Tingey, and R. A. Reinert. 1975. Oxides of nitrogen. Pages 121-139. In: J. B. Mudd and T. T. Kozlowski eds. Responses of plants to air pollution. Academic Press, NY.

(41) U.S. Environmental Protection Agency. 1979. Air quality criteria for oxides of nitrogen. EPA 600/8-82-026. Environmental Criteria and Assessment Office, Office of Research and Development, U.S. EPA, Research Triangle Park, NC.

(42) U.S. Environmental Protection Agency. 1979. Air quality criteria for carbon monoxide. EPA 600/8-79-022. Environmentl Criteria and Assessment Office. Office of Research and Development, U.S. EPA, Research Triangle Park, NC.

(43) Steubing, E. W., R. H. Frickel, and G. O. Rubel. 1980. Recent research on phosphorus smoke. AD No. A090422. Chemical Systems Laboratory, Aberdeen Proving Ground, MD.

(44) Ausmus, B. S., G. J. Dodson, and D. R., Jackson, 1978. Behavior of heavy metals in forest microcosms. III. Effects on litter-soil carbon metabolism. Water Air Soil Pollut. 10:19-26.

(45) Jackson, D. R., C. D. Washburne, and B. S. Ausmus. 1977. Loss of Ca and NO_3-N from terrestrial microcosms as an indicator of soil pollution. Water Air Soil Pollut. 8:279-284.

(46) Foth, H. D. 1978. Fundamentals of soil science. 6th Ed. [illegible] & Sons, New York, NY. 436 pp.

(47) Liebig, G. F. 1965. Chapter 2: Arsenic. Pages 13-23. In: H. D. Chapman, ed.. Diagnostic criteria for plants and soils. Quality Printing Company, Inc., Abilene, TX.

(48) Chapman, H. D. 1965. Diagnostic criteria for plants and soils. Quality Printing Company, Inc., Abilene, TX.

(49) Dvorak, A. J., and B. G. Lewis, eds. 1978. Impacts of coal-fired power plants on fish, wildlife, and their habitats. U.S. Department of the Interior, Fish and Wildlife Service, Office of Biological Services, FWS/OBS-78/29, Washington, DC. 260 pp.

(50) Allaway, W. H. 1968. Agronomic controls over the environmental cycling of trace elements. Advances in Agron. 20:235-274.

(51) Charg. F.-H. and M. Alexander. 1983. Effects of simulated acid precipitation on growth and nodulation of leguminous plants. Bull. Environ. Contam. Toxicol. 30:379-387.

(52) Pratt, P. F. 1965. Chapter 1: Aluminum. Pages 3-12. In: H. D. Chapman, ed. Diagnostic criteria for plants and soils. Quality Printing Company, Inc., Abilene, TX.

(53) McFee, W. W. 1980. Sensitivity of soil regions to acid precipitation. EPA-600/3-80-013. Environmental Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Corvallis, OR.

(54) McFee, W. W. 1983. Sensitivity ratings of soils to acid deposition: A review. Environ. Exp. Bot. 23: 203-210.

(55) Brewer, R. F. 1965. Chapter 16: Lead. Pages 213-217. In: H. D. Chapman, ed. Diagnostic criteria for plants and soils. Quality Printing Company Inc., Abilene, TX.

(56) Duke, K. M., M. E. Davis, and A. J. Dennis. 1977. IERL-RTP procedures manual: Level 1 environmental assessment biological tests for pilot studies. EPA-600/7-77-043. Industrial Environmental Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC.

APPENDIX A

EXPOSURE PROTOCOL AND DATA

10. Prior to injecting the sample into the dilution box, a second stop watch should be handy and the fan inside the box should be turned on. As the appropriate amount of sample is being injected into the dilution box the stop watch should be started. This "time" refers to the "read time" on the Data Sheet and is the time past injection up to the moment when the mass monitor reading is taken.

11. With concentration being displayed, record the reading and press CHK and START buttons. If the frequency displayed does not exceed an acceptable level you may repeat the measurement. Otherwise the sensor should be cleaned. Record "Read Time" every time you start a new measurement.

12. After each sample has been measured with the mass monitor, the syringe is removed from the tygon tube and the dilution box is evacuated by hooking a vacuum pump up to the syringe port.

Data Sheet

13. Data sheet (see attached form) should be filled out during the test as follows:

    - o Combustion Time - start to end of combustion of phosphorus (min', s").
    - o RH - relative humidity of the room and chamber atmosphere should be taken at run time zero and every 0.5 hour during test (%).
    - o Temp - temperature in a chamber should be recorded at time 0, several times during combustion, and every 0.5 hr. after that (^{o}F).
    - o Sample # refers to a gas sample taken from an exposure chamber.
    - o Run Time - time elapsed from the ignition of the phosphorus (min', s").
    - o Read Time - time elapsed from the moment of sample injection into the dilution box (min', s").
    - o Volume Dispensed - gas volume injected into the dilution box (cc).
    - o Measurement Time - duration of the reading procedure (24 or 120 seconds).
    - o Reading - final reading displayed at the end of the measurement cycle (mg/m^3).

Exhaust

14. Exhaust process begins at the end of the 2-hr. exposure period. Both an exhaust hose and a port on the chamber top should be unplugged and connected to each other just prior to the end of the exposure. Exhaust

blower attached to exhaust line outside of the test room is turned on at the appropriate time and left on for about ten minutes. To prevent damage of exposure chamber, make sure to unplug opening on the front wall of chamber so that room air is pulled tnrough it.

15. Repeat the exhaust procedure for all exposure chambers. After this process is completed, plug up the exhaust ports and flexible hoses with caps, lift hoods off the chambers and start preparation for the next tests. Turn off the exposure lights and agitating fans. Make sure the deposition coupons are labeled correctly and sealed witn tape.

Test # _____ Date __/__/____ Operators _____

Chamber # _____ Target Concentration (mg/m^3) _____

Cart # _____ Material _____

Deposition Coupon # _____ Amount _____

Actual Time _____

Combustion Time _____

Run Time	RH (%)		Temp (°F)	
	Room	Chamber	Room	Chamber

Sample #	TSI 3500 # (1 or 2)	TSI 3500 Run Time	Read Time	Measurement Time (24 or 120)	Volume Dispersed (ml)	Reading (mg/m^3)	Theoret Value (mg/m^3)	Corr (mg/m^3)	Comments

Comments: _____

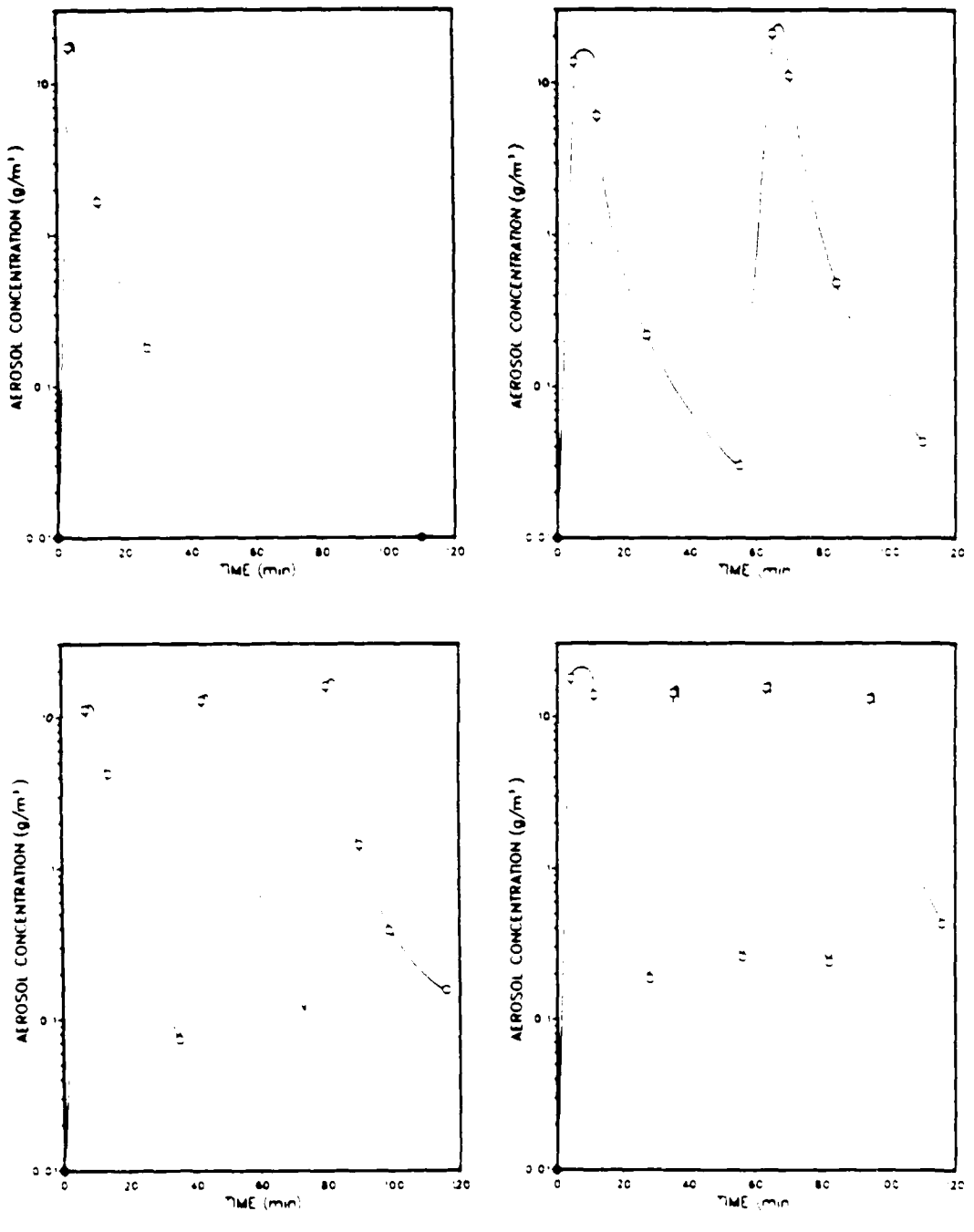


FIGURE A-1. AEROSOL MASS CONCENTRATION FOR PRELIMINARY EXPOSURE TO RP-80 SMOKE AT THE [illegible] AND THREE [illegible] AND FOUR [illegible] CONCENTRATIONS OF RP-80 MATERIAL

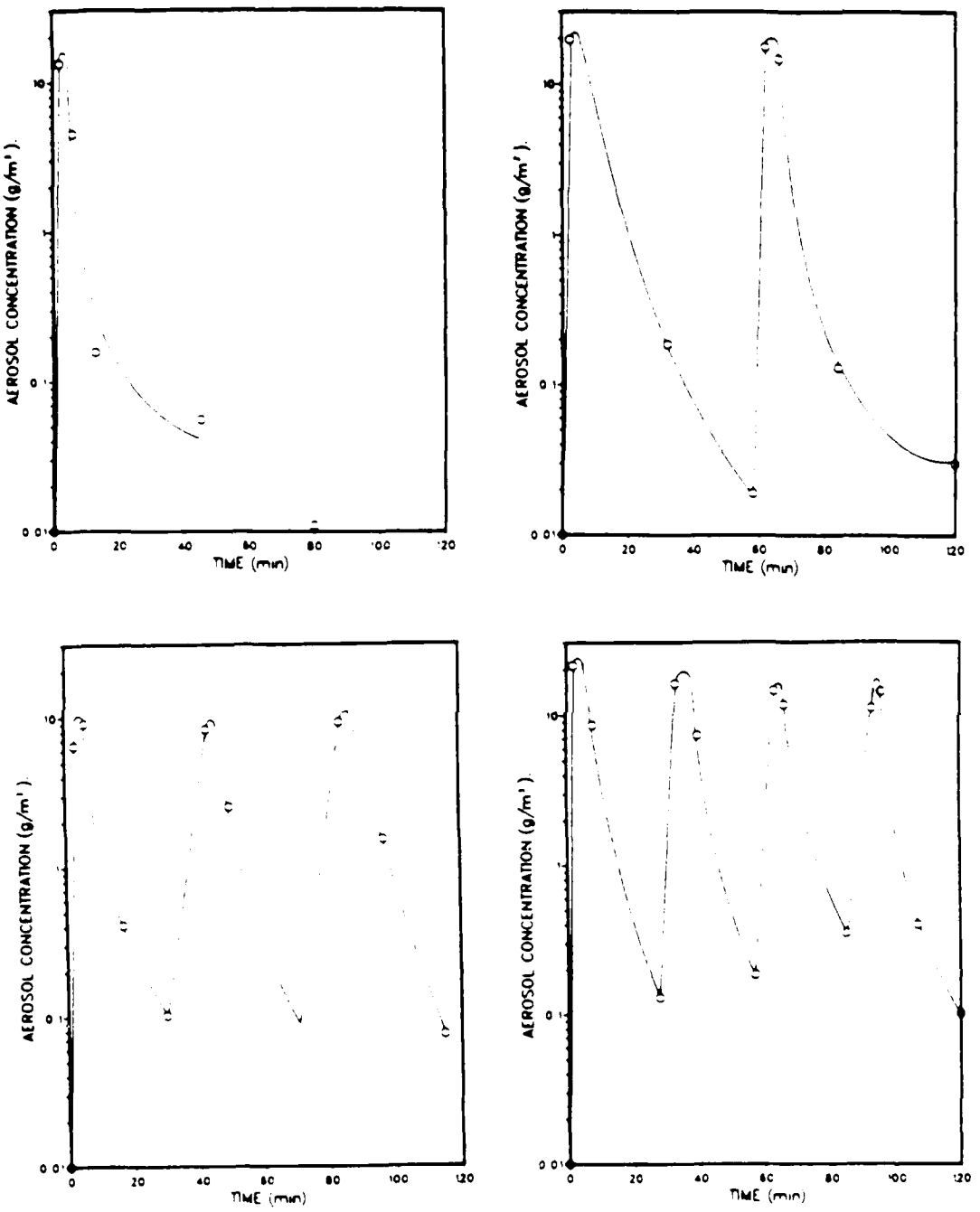


FIGURE A-2. AEROSOL MASS CONCENTRATIONS FOR PRELIMINARY EXPOSURE TO WP FUMO E AT TIME (2a), TWO (2b), THREE (2c), AND FOUR (2d) IGNITIONS OF THREE PELLETS OF WP MATERIAL

TABLE A-1. AVERAGE TEMPERATURE AND RELATIVE HUMIDITY FOR RP/BR TESTS

Test Number	Group A Target Conc, mg/m^3								Group B Target Conc, mg/m^3							
	100		300		600		1500		100		300		600		1500	
	T, C	RH, %	T, C	RH, %	T, C	RH, %	T, C	RH, %	T, C	RH, %	T, C	RH, %	T, C	RH, %	T, C	RH, %
1	27	NA	27	NA	29	NA	31	NA	22	72	22	78	22	77	24	66
2	24	NA	23	NA	24	NA	26	NA	25	71	25	66	26	79	27	64
3	27	65	26	65	28	76	29	62	25	74	26	74	28	82	28	75
4	25	68	25	69	25	71	26	64	29	65	28	65	29	64	31	58
5	30	69	30	69	30	65	31	64	26	70	27	72	28	62	29	58
6	31	69	31	68	31	64	32	61	28	54	29	55	33	56	30	39
7	23	73	23	78	23	68	24	65	26	65	26	63	26	60	28	58
8	23	69	23	70	24	66	26	55	29	62	29	61	30	56	32	48
9	31	60	32	60	32	55	34	45	33	64	34	62	34	58	35	58
10	33	64	33	66	34	56	35	49	33	68	33	68	33	64	34	59
11	33	61	33	38	35	51	35	48	36	48	36	51	38	32	38	49
12	35	59	35	60	36	43	38	53	32	61	32	62	34	54	35	64
13	35	57	35	52	36	37	37	54	26	88	25	71	25	84	26	85
14	33	72	33	73	35	65	36	59	30	85	30	80	33	75	33	79
15	31	68	31	57	32	52	33	40	30	81	30	78	30	75	31	71
16	31	69	32	66	32	52	34	33	31	82	31	75	33	62	33	51

TABLE A-2. AVERAGE TEMPERATURE AND RELATIVE HUMIDITY FOR WP TESTS

Test Number	Group A Target Conc, mg/m^3								Group B Target Conc, mg/m^3							
	100		300		600		1500		100		300		600		1500	
	T, C	RH, %	T, C	RH, %	T, C	RH, %	T, C	RH, %	T, C	RH, %	T, C	RH, %	T, C	RH, %	T, C	RH, %
1	25	NA	25	NA	27	NA	27	NA	22	NA	22	NA	22	71	27	76
2	22	NA	23	NA	23	NA	26	NA	23	NA	22	81	24	82	26	77
3	20	79	22	80	23	80	24	77	22	70	22	70	22	73	25	67
4	22	68	24	69	25	71	26	64	24	71	24	72	24	70	26	67
5	25	75	25	76	26	77	27	72	23	81	23	83	24	77	24	71
6	26	77	26	77	27	76	28	73	22	72	23	70	23	69	26	63
7	21	76	21	76	21	73	22	70	23	72	23	71	23	69	24	62
8	21	73	22	77	22	71	23	67	24	71	23	69	24	70	25	65
9	26	71	25	71	26	66	27	61	28	77	29	79	30	72	31	67
10	26	70	26	69	27	64	29	60	27	78	28	81	29	75	31	73
11	27	76	27	60	28	66	29	71	30	79	30	81	32	56	32	73
12	26	75	26	78	28	59	30	73	28	79	28	86	29	68	29	80
13	30	75	29	77	30	63	32	76	26	79	26	84	27	76	27	76
14	28	81	28	85	30	80	30	78	24	89	25	87	25	84	26	85
15	24	84	24	78	26	71	27	72	25	70	25	81	26	75	26	73
16	23	83	23	76	25	68	26	68	25	86	25	82	26	77	27	77

APPENDIX B

ECOLOGICAL DATA

TABLE B-1. LOSS OF TOC AND K IN THE FIRST LEACHATE FROM MICROCOSMS EXPOSED TO RP/BR OR WP SMOKE

Nutrient	Target Dose (mg/m^3)	Weight (µg) of Nutrient Lost in Leachate			
		RP/BR Smoke		WP Smoke	
		Mean	±Std. Dev.(c)	Mean	±Std. Dev.(c)
TOC(a)	0	8.00	0.71	7.65	1.77
	100	6.75	1.77	8.15	0.92
	300	7.65	1.20	8.25	2.05
	600	8.20	1.13	6.55	1.91
	1500	9.05	0.92	9.25	1.20
K(b)	0	3222.00	1094.60	2410.00	1145.51
	100	3321.00	2227.39	2354.00	828.73
	300	2099.00	284.26	3344.00	161.22
	600	3550.00	1566.95	2569.50	939.74
	1500	4413.75	2578.46	3053.00	793.37

(a) TOC = total organic carbon; in this case TOC is assumed to equal dissolved organic carbon, because leachate was filtered thorugh a 0.45 µ filter.

(b) K = potassium.

(c) Std. Dev. = standard deviation.

TABLE B-2. LOSS OF Ca AND NO_3-N ON THREE SEPARATE LEACHING DATES FROM MICROCOSMS EXPOSED TO RP/BR SMOKE

Leachate	Target Dose (mg/m^3)	Weight (µg) of Nutrient Loss in Leachate			
		Ca(a)		NO_3-N(b)	
		Mean	± Std. Dev.	Mean	± Std. Dev.
First	0	28272.50	6325.07	156.65	202.30
	100	14321.50	6672.97	11.65	2.76
	300	11763.75	4506.04	9.40	2.97
	600	12896.25	6464.72	10.55	4.45
	1500	28112.50	3676.96	17.30	2.26
Second	0	20771.25	5467.70	55.80	47.94
	100	12248.75	7755.19	68.53	62.12
	300	15255.00	8407.50	25.10	12.87
	600	11658.75	1702.36	33.30	32.24
	1500	24207.50	14311.84	68.55	15.63
Third	0	13822.50	1276.33	41.67	39.70
	100	11882.50	2549.12	8.35	5.02
	300	9297.50	1325.83	8.85	0.07
	600	12900.00	3867.87	11.25	0.78
	1500	34552.50	10977.83	18.70	0.14
Cumulative Sum	0	62866.25	13069.10	254.12	194.07
	100	38452.75	16977.28	88.53	69.90
	300	36316.25	2575.64	43.35	15.91
	600	37455.00	8630.24	55.10	37.48
	1500	86872.50	7010.96	104.55	18.03

(a) Ca = Calcium.
(b) NO_3-N = Nitrate-Nitrogen.

TABLE B-3. LOSS OF Ca AND NO_3-N ON THREE SEPARATE LEACHING DATES FROM MICROCOSMS EXPOSED TO WP SMOKE

Leachate	Target Dose (mg/m^3)	Weight (µg) of Nutrient Loss in Leachate			
		Ca(a)		NO_3-N(b)	
		Mean	±Std. Dev.	Mean	±Std. Dev.
First	0	10810.00	975.81	97.00	123.04
	100	13872.50	130.81	45.00	19.52
	300	15853.75	5891.97	13.35	6.86
	600	10810.00	1774.84	60.25	67.25
	1500	15521.75	6847.27	11.40	3.54
Second	0	24786.25	17912.78	72.28	2.23
	100	17475.00	5536.65	29.50	22.20
	300	30943.75	836.15	22.55	1.77
	600	14355.00	2877.92	89.48	31.50
	1500	18653.75	1833.17	27.55	20.44
Third	0	21175.00	8414.57	15.00	0.71
	100	19845.00	1732.41	15.25	2.76
	300	33208.75	17166.78	17.95	1.06
	600	12171.25	8600.19	11.65	4.60
	1500	29157.00	10441.14	22.60	4.95
Cumulative Sum	0	56771.25	25351.55	184.28	125.97
	100	51192.50	7138.24	89.75	38.96
	300	80006.25	10438.66	53.85	7.57
	600	37336.25	9703.27	161.38	103.34
	1500	63332.50	5427.04	61.55	21.85

(a) Ca = Calcium.
(b) NO_3-N = Nitrate-Nitrogen.

TABLE B-4. BIOMASS(a) YIELD FROM FIRST HARVEST OF MICROCOSMS EXPOSED TO RP/BR OR WP SMOKE

Smoke	Target Dose (mg/m^3)	Biomass (g)	
		Mean	± Std. Dev.
RP/BR	0	18.57	0.03
	100	18.24	0.09
	300	19.12	0.97
	600	20.00	2.15
	1500	18.59	0.05
WP	0	19.66	3.01
	100	18.72	0.50
	300	19.52	2.40
	600	19.72	0.14
	1500	18.15	0.05

(a) Above-ground dry weight.

TABLE B-5. BIOMASS(a) YIELD FOR INDIVIDUAL SPECIES FROM THE SECOND HARVEST OF MICROCOSMS EXPOSED TO RP/BR OR WP SMOKE

Smoke	Target Dose (mg m^{-3})	Biomass(g) Wheat Mean	Wheat ±Std. Dev.	Ryegrass Mean	Ryegrass ±Std. Dev.	Sweetclover Mean	Sweetclover ±Std. Dev.
RP/BR	0	6.44	0.63	0.48	0.14	3.71	1.48
	100	7.03	0.72	0.39	0.20	2.92	0.45
	300	6.17	0.38	0.49	0.14	4.50	0.49
	600	6.17	0.98	0.58	0.18	5.03	1.83
	1500	7.49	1.66	0.66	0.06	1.90	0.33
WP	0	6.72	1.06	0.59	0.48	4.55	0.05
	100	6.56	0.45	0.64	0.10	4.81	0.43
	300	6.31	1.71	1.04	0.28	6.13	0.26
	600	5.59	0.57	0.44	0.05	5.75	0.52
	1500	8.70	0.63	0.58	0.06	1.61	0.54

(a) Above-ground dry weight.

TABLE B-6. GRAND TOTAL BIOMASS[a] YIELD FOR ALL SPECIES FROM BOTH HARVESTS OF MICROCOSMS EXPOSED TO RP/BR OR WP SMOKE

Smoke	Target Dose (mg/m^3)	Biomass (g) Mean	± Std. Dev.
RP/BR	0	29.20	0.97
	100	28.58	0.15
	300	30.48	1.98
	600	31.88	3.18
	1500	28.63	1.33
WP	0	31.52	3.67
	100	30.73	1.28
	300	33.00	3.57
	600	31.50	0.89
	1500	28.94	1.14

(a) Above-ground dry weight.

TABLE B-7. ELEMENT CONCENTRATIONS (µg/g) IN PLANT TISSUE FROM THE FIRST HARVEST OF MICROCOSMS EXPOSED TO RP/BR SMOKE

	Target Dose (mg/m³)									
	0		100		300		600		1500	
Element	Mean	± Std. Dev.	Mean	± Std. Dev.	Mean	± Std. Dev.	Mean	± Std. Dev.	Mean	± Std. Dev.
Aluminum	12.55	8.56	9.00	0.99	7.45	3.61	6.15	1.63	[illegible]	[illegible]
Arsenic	0.08	0.04	0.10	0.00	0.20	0.00	0.45	[illegible]	[illegible]	[illegible]
Boron	51.90	0.00	65.60	25.60	49.65	9.40	63.40	[illegible]	[illegible]	[illegible]
Barium	13.30	2.55	27.95	17.18	15.60	0.42	13.15	[illegible]	[illegible]	[illegible]
Calcium	10234.50	2604.70	9042.85	3368.02	8343.75	1352.34	8898.80	[illegible]	[illegible]	[illegible]
Cadmium	0.20	0.00	0.20	0.00	0.20	0.00	0.20	[illegible]	[illegible]	[illegible]
Cobalt	0.30	0.00	0.30	0.00	0.30	0.00	0.30	[illegible]	[illegible]	[illegible]
Chromium	0.20	0.00	0.30	0.14	0.20	0.00	0.25	[illegible]	[illegible]	[illegible]
Copper	5.50	0.14	5.45	0.64	5.45	0.64	5.35	[illegible]	[illegible]	[illegible]
Iron	216.40	181.02	90.80	30.69	51.30	3.54	51.90	[illegible]	[illegible]	[illegible]
Lead	0.60	0.00	1.50	0.14	1.10	0.71	0.60	[illegible]	[illegible]	[illegible]
Magnesium	3661.90	365.01	3708.50	452.12	3462.50	350.02	3576.90	[illegible]	[illegible]	[illegible]
Manganese	31.10	2.97	38.40	0.85	33.85	0.07	31.60	[illegible]	[illegible]	[illegible]
Molybdenum	8.70	0.14	9.45	0.64	8.35	1.06	8.35	[illegible]	[illegible]	[illegible]
Nickel	1.05	0.64	[illegible]	[illegible]	0.80	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Phosphorus	1464.10	124.17	1774.10	133.93	2116.90	36.20	[illegible]	[illegible]	[illegible]	[illegible]
Selenium	0.05	0.00	0.05	0.00	0.05	0.00	[illegible]	[illegible]	[illegible]	[illegible]
Sodium	585.65	239.50	1016.90	294.30	843.15	32.74	[illegible]	[illegible]	[illegible]	[illegible]
Strontium	9.55	1.06	9.20	3.11	8.75	0.49	[illegible]	[illegible]	[illegible]	[illegible]
Titanium	1.00	0.00	1.00	0.00	1.00	0.00	[illegible]	[illegible]	[illegible]	[illegible]
Thallium	2.15	0.21	2.15	0.21	2.00	0.00	[illegible]	[illegible]	[illegible]	[illegible]
Vanadium	0.20	0.00	0.50	0.14	0.35	0.21	[illegible]	[illegible]	[illegible]	[illegible]
Yttrium	0.35	0.07	0.50	0.14	0.40	0.14	[illegible]	[illegible]	[illegible]	[illegible]
Zirconium	15.55	1.77	17.15	0.49	16.10	1.13	[illegible]	[illegible]	[illegible]	[illegible]

TABLE B-8. ELEMENT CONCENTRATIONS (µg/g) IN PLANT TISSUE FROM THE FIRST HARVEST OF MICROCOSMS EXPOSED TO WP SMOKE

	Target Dose (mg/m^3)									
	0		100		300		600		1500	
Element	Mean	± Std. Dev.	Mean	± Std. Dev.	Mean	± Std. Dev.	Mean	± Std. Dev.	Mean	± Std. Dev.
Aluminum	11.40	4.38	5.85	4.17	4.60	1.98	17.05	4.11	7.90	1.27
Arsenic	0.05	0.04	0.05	0.00	0.10	0.00	0.25	[illegible]	0.60	[illegible]
Boron	43.65	4.03	44.05	3.18	70.55	25.81	65.65	4.39	51.15	14.35
Barium	13.6	1.95	14.7	1.27	17.95	0.07	16.55	[illegible]	15.6	[illegible]
Calcium	8104.40	2276.14	8987.65	1810.19	9154.10	359.35	9351.25	1709.39	7971.30	1665.24
Cadmium	0.20	0.00	0.20	0.00	0.20	0.00	0.20	[illegible]	[illegible]	[illegible]
Cobalt	0.45	0.21	0.35	0.00	0.30	0.00	0.35	[illegible]	0.35	[illegible]
Chromium	0.20	[illegible]	0.20	0.00	0.40	0.28	0.20	[illegible]	[illegible]	[illegible]
Copper	6.35	[illegible]	6.35	0.07	6.80	0.71	6.30	[illegible]	6.35	0.07
Iron	46.65	9.26	45.05	1.77	53.10	1.84	48.15	6.15	46.90	2.69
Lead	[illegible]	[illegible]	0.60	0.00	0.60	0.00	2.65	[illegible]	[illegible]	[illegible]
Magnesium	3041.90	831.70	3753.15	706.19	3921.60	12.87	3919.40	68.10	3662.65	285.19
Manganese	32.65	[illegible]	34.05	1.34	37.25	1.34	36.25	[illegible]	35.15	6.15
Molybdenum	8.15	0.92	10.30	1.41	9.15	0.49	9.40	0.85	[illegible]	0.92
Nickel	0.55	[illegible]	0.35	0.00	0.40	0.14	0.55	[illegible]	[illegible]	[illegible]
Phosphorus	[illegible]	[illegible]	1836.90	291.16	2662.8	144.11	3952.65	3.04	[illegible]	[illegible]
Selenium	[illegible]	[illegible]	[illegible]	0.00	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Silicon	[illegible]	41.96	927.90	224.01	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Strontium	[illegible]	[illegible]	8.65	0.21	9.85	1.21	9.35	[illegible]	8.35	2.61
Titanium	[illegible]	[illegible]	[illegible]	0.00	[illegible]	0.00	[illegible]	[illegible]	[illegible]	[illegible]
Thallium	2.30	0.42	2.90	1.27	2.00	0.00	3.55	0.35	2.10	[illegible]
Vanadium	[illegible]	0.35	0.30	0.14	0.20	0.00	1.00	0.57	[illegible]	0.00
Yttrium	[illegible]	[illegible]	0.90	0.85	0.30	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Zinc	14.20	0.57	17.10	1.13	20.00	1.41	18.70	[illegible]	19.15	3.04

TABLE B-9. ELEMENT CONCENTRATIONS (µg/g) IN PLANT TISSUE FROM THE SECOND HARVEST OF MICROCOSMS EXPOSED TO RP/BR OR WF SMOKE

		Target Dose (mg/m^3)									
		0		100		300		600		1500	
Smoke	Element	Mean	± Std. Dev.	Mean	± Std. Dev.	Mean	± Std. Dev.	Mean	± Std. Dev.	Mean	± Std. Dev.
RP/BR	Aluminum	13.90	3.96	16.15	2.62	14.35	2.47	18.50	2.26	17.25	5.73
	Arsenic	0.05[a]	0.00	0.07	0.03	0.30	0.00	0.65	0.00	2.65	0.49
	Chromium	3.25	3.46	1.00	0.57	1.30	0.71	1.55	0.00	2.40	1.13
	Lead	7.15	0.21	6.70	2.55	6.70	2.97	10.15	1.06	9.60	1.84
	Molybdenum	5.20	6.65	10.85	4.03	7.70	0.57	10.05	1.45	2.00	6.36
	Phosphorus	2428.20	322.72	3312.95	1093.12	4057.05	204.42	7476.65	117.59	15287.50	2246.76
WF	Aluminum	18.25	2.47	18.45	0.64	26.00	4.10	19.75	4.03	28.30	3.39
	Arsenic	0.13	0.11	0.13	0.11	0.15	0.07	0.15	0.00	0.90	0.28
	Chromium	0.95	0.21	1.85	1.48	1.70	0.57	1.25	0.90	2.15	1.20
	Lead	7.75	0.35	9.00	1.56	14.20	1.13	10.00	4.95	15.70	0.99
	Molybdenum	9.25	1.20	10.30	0.71	11.15	1.06	9.15	1.77	11.25	1.20
	Phosphorus	2118.80	176.78	2654.40	128.13	5326.90	2194.72	4569.40	1130.58	12850.00	1162.48

[a] Detection limit has been set at 0.05 µg/g; values less than that have been set to 0.05.

TABLE B-10. LEAF SURFACE AREA BY SPECIES OF PLANTS FROM THE SECOND HARVEST OF MICROCOSMS EXPOSED TO RP/BP OR WP SMOKE

Smoke	Target Dose (mg m^{-3})	Leaf Surface Area (cm^2)					
		Wheat		Ryegrass		Sweet Clover	
		Mean	± Std. Dev.	Mean	± Std. Dev.	Mean	± Std. Dev.
RP/BP	0	520.00	75.42	82.50	16.26	460.17	150.61
	100	614.67	33.47	60.17	27.11	390.67	63.64
	300	584.67	32.53	72.17	24.75	678.17	48.32
	600	580.50	6.84	82.33	29.70	770.17	261.39
	1500	631.50	105.36	83.17	16.73	521.33	70.24
WP	0	585.83	38.89	85.83	74.25	517.00	15.08
	100	763.17	43.13	115.67	16.50	635.00	230.99
	300	680.17	132.70	178.83	27.11	925.67	36.77
	600	674.00	33.47	81.17	10.14	959.67	220.62
	1500	807.67	112.19	84.33	27.34	296.50	73.77

TABLE B-11. LIGHT INTENSITY (MICROEINSTEINS PER SQUARE METER PER SECOND) IN EXPOSURE CHAMBERS AS A FUNCTION OF SMOKE CONCENTRATION DUE TO RP/BR OR WP COMBUSTION

Concentration:	0 mg/m^3	100 mg/m^3					300 mg/m^3			60 mg/m^3				1500 mg/m^3			
		WP			RP/BR		WP	RP/BR		WP		RP/BR		WP		RP/BR	
Date:	6/8	6/13	6/18	6/28	6/14	6/27	6/27	6/13	6/26	6/12	6/26	6/11	6/25	6/19	6/25	6/5	6/19
Time (min)																	
0 (lights off)		--	100	24	39	32	10	--	69	27	77	--	27	--	25	30	40
0 (lights on)		--	--	135		155	150	160	198	220	240	180	165	--	165	200	180
1	45	62	120	115	47	115	105	95	150	80	75	165	85	75	65	130	75
5	100	97	120	120	140	130	110	120		97	90	145	88	--	71	77	105
10 (end combustion)		115	120	120	160	135	115	135	150	110	105	150	110	83	71	77	105
20-60	115	133	125	120	160	138	120	170	157	150	122	170	123	109	95	145	122
90-120	110	160	143	123	150	135	125	145	178	180	170	173	130	125	130	175	135

PUBLICATIONS AND PERSONNEL

The following presentations were made under Contract DAMD17-84-C-4001:

Arthur, M. F., D. A. Tolle, and T. C. Zwick. 1984. Use of soil microbial respiration for evaluating materials for land application. Poster presentation at Fifth Annual Meeting, Society of Environmental Toxicology and Chemistry, Nov. 4-7, 1984, Hyatt Regency Crystal City Hotel, Arlington, VA.

Duke, K. M., D. A. Tolle, and T. C. Zwick. 1984. Plant stress ethylene: An effective screening bioassay. Poster presentation at Fifth Annual Meeting, Society of Environmental Toxicology and Chemistry, Nov. 4-7, 1984, Hyatt Regency Crystal City Hotel, Arlington, VA.

Tolle, D. A., M. F. Arthur, K. M. Duke, J. Chesson, M. R. Kuhlman. 1984. Terrestrial microcosm evaluation of dose-response relationships due to exposure by two phosphorus obscurant smokes. Presented at Fifth Annual Meeting, Society of Envirionmental Toxicology and Chemistry, Nov. 4-7, 1984, Hyatt Regency Crystal City Hotel, Arlington, VA.

The following personnel participated in the phosphorus smoke evaluation:

Professional Staff

Duane A. Tolle, Principal Investigator
Michael R. Kuhlman, Task 1 Leader
Mickey F. Arthur, Task 2 Leader
Danny R. Jackson, Task 3 Leader
Jean Chesson, Task 4 Leader
Kenneth M. Duke, Program Manager
L. Barry Goss, Section Manager
Vladimir Kogan
Paul I. Feder

Dean P. Margeson
Jeffery L. Means
Karen S. Rush
Richard D. Foltz
Bruce W. Vigon
Charles T. Litsey
M. Claire Matthews

Technicians

Thomas C. Zwick
Peter J. Ferron
G. Kelly O'Brien
Lisa C. Hendrickson
Mark S. Ross
Donald L. Sgontz
Sandra L. Clark
James R. Dixey
Amanda J. Osburn
Gregory L. Headington
Lawrence W. Miga
Robert E. Zimmerman
Harry G. Leonard
Fred R. Moore

DISTRIBUTION LIST

24 copies	Commander ATTN: SGRD-UBZ US Army Medical Bioengineering Research and Development Laboratory Fort Detrick, Frederick, MD 21701-5010
1 copy	Commander US Army Medical Research and Development Command ATTN: SGRD-RMI-S Fort Detrick, Frederick, MD 21701-5012
12 copies	Defense Technical Information Center (DTIC) ATTN: DTIC-DDAC Cameron Station Alexandria, VA 22304-6145
1 copy	Dean School of Medicine Uniformed Services University of the Health Sciences 4301 Jones Bridge Road Bethesda, MD 20814-4799
1 copy	Commandant Academy of Health Sciences, US Army ATTN: AHS-CDM Fort Sam, Houston, TX 78234-6100
1 copy	Commander US Army Medical Bioengineering Research and Development Laboratory ATTN: SGRD-UBZ-T Librarian Fort Detrick, Frederick, MD 21701-5010
1 copy	HQDA ATTN: DAEN-RDM/Dr. Meyer 20 Massachusetts Avenue, NE Washington, DC 20314

END

DATE

FILMED

5-88

DTIC